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PHYSIOLOGICAL FACTORS NECESSARY TO ALLEVIATE GENETIC LETHAL ANEMIA IN MICE

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THE fact that certain pathological conditions have been shown to be due to definite gene complexes and that these pathological conditions may be eliminated from a strain by the substitution of another equally simple gene complex for the one at fault, suggests that other agents, taken possibly from the environment, could also supplement the unfavorable inheritance to produce a normal individual out of what is potentially a bad inheritance. This paper is based on an inquiry into this problem in the case of a single pathological condition, the lethal anemia of mice, found associated with one gene, the adult character of which is dominant white spotting.

The existence of this gene was first brought to light by Little (1), in 1915, when he indicated that two genes for this dominant white, when present in the same individual, were lethal in their effect. This lethal factor generally kills the animal at or shortly after birth, as both Delfsen and De Aberle (2, 3) have shown. It is usually difficult to find the young since the mother eats them either because they are obviously weaklings or because they are dead. The young anemics, when obtained alive, are distinctive in appearance. They are generally smaller than the normal individuals of the litter and present a dead white and bloodless appearance. The

anemics often have vigorous appetites and suck large quantities of milk. They are, however, unable to utilize this milk for their proper nutrition due to the presence of this gene, and usually die before the 7th day. If the animal is autopsied the organs, especially those normally having a large amount of blood, are pale in color.

In our investigations, heterozygous matings of the black-eyed white mice carrying the recessive anemic factor produced 103 anemic young mice in 903 births, or 11.4 per cent. Not all these matings were checked every day. In 180 births where the matings were examined morning and night or oftener the number of anemics was 40, or 22.2 per cent.

TABLE I
ANEMIC BIRTHS AND NORMAL BIRTHS OBSERVED IN ALL LITTERS (1),
AND IN THOSE EXAMINED EVERY 24 HOURS OR LESS (2)

	Anemics	Normals	Per cent. anemics of total
(1) All births	103	903	11.4 \pm 0.7
(2) Births less than 20 hours old when ex- amined	40	160	22.2 \pm 2.1

Under these more controlled conditions the results therefore approach those expected of a simple Mendelian ratio, 25 per cent. Our data consequently conform to those of Dr. De Aberle, in which she showed that for 500 young, 17 per cent. anemics were obtained when the cages were inspected two or three times a day and only 10 per cent. when inspection was delayed for 24 hours or over. Dr. De Aberle further showed that when 16-day-old fetuses were examined *in utero*, the percentage of anemics was 24.6 per cent., practically an exact Mendelian ratio. The fact that the death rate of the fetuses in the uteri of the parents which genetically could produce only normal young was only slightly greater than that for the parents which produced anemics showed that no fetal nutritional factor, fetal congestion or like cause

can be invoked as the primal factor in the production of the anemic progeny. The fate of these young was sealed at the fertilization of the egg. The anemia was a matter of heredity. This conclusion is further borne out by the distribution of anemics in litters of different sizes when the litters were examined every 24 hours.

TABLE II
DISTRIBUTION OF ANEMIC BIRTHS WITH LITTER SIZE

No. of young in litter	No. of Anemics					Total litters
	0	1	2	3	4	
1.....	1					1
2.....	6	1				7
3.....	6	1				7
4.....	5	6	1			12
5.....	4	5	2			11
6.....	3	8	3	1		15
7.....		2	2			4
8.....	2	1		2		5
9.....						
10.....	1					1
11.....				1	1	2
Total.....	28	24	8	4	1	65

The genes for this lethal anemia are an integral part of each daughter cell coming from the fertilized egg. The data obviously suggest the important problem, is it possible to replace artificially these gene effects as manifested by a deficiency of necessary products and, by so doing, enable the animal to react normally in spite of the presence in its cells of these unfavorable genes? The end somatic reaction produced by these genes is an animal which weighs at birth about 68 per cent. of the normal weight. The dried weight of the normal mouse is 18 per cent. of the birth weight. The dried weight of the anemic

mouse is 14 per cent. of the birth weight. There is consequently relatively less solid in the anemic mouse than in the normal. The blood has a one third to one fourth of the hemoglobin found in a heterozygous black-eyed white parent and the red cell count is correspondingly reduced. The heterozygous black-eyed white mice, litter mates of the anemics, have only about three fourths of the hemoglobin found in mice of the same genetic constitution but three months old. The hemoglobin of the anemic appears to be similar to that found in the normal, according to our colleague, Dr. Anson, who was kind enough to test the carbon monoxide and oxygen bands of the hemoglobin spectra. Since the red cell count of anemics is reduced to one third to one fourth of that of their normal mates, the anemia appears to be due to lack of red cell formation rather than to an improper proportion of hemoglobin in the cells.

TABLE III

Character	Anemic	Black-eyed white mouse
Birth weight	$0.97 \pm .03$ gms.	$1.39 \pm .05$ gms.
Dried birth weight	$0.13 \pm .01$	$0.25 \pm .02$
Hemoglobin per 100 cc	4.2	12.5
Fe-mg. per mouse	$0.08 \pm .01$	$0.10 \pm .01$
Fe-mg. per gms. of dry weight	$0.56 \pm .07$	$0.43 \pm .08$

The known relation of iron to hemoglobin formation led to the determination of that constituent of the body in the belief that the mechanism of iron metabolism might have been upset by this gene. The whole mouse was dissolved and the organic matter destroyed by concentrated HNO_3 and H_2SO_4 with a small amount of sodium chlorate added at the end of the digestion to remove the last traces of organic matter. The residue, when evaporated to dryness, was taken up in 5 cc of weak HCl for analysis by the method of Wong (4). The

amount of iron was then converted into milligrams of iron per gram of dry weight. The analytical results were irregular, the variation between mice being large. The material shows that the anemic mice contained at least as much iron as the controls when compared on a dry weight basis. This conclusion was to be expected since we are dealing with the newly born animal when the materials found in its body are those directly deposited in it from the mother's circulation. Milk is deficient in iron and most animals which undergo a nursing period have sufficient iron stored in their bodies from their dam to supply the needs of this period. The anemic mouse does not differ from the normal in this regard.

Since anemias are found in the uteri in the Mendelian proportion of 1 anemic fetus to 3 not anemic, it is evident that the principle which prevents the anemia can not pass the placenta from the mother's blood to the anemic's blood, or that the anemic has a substance in its blood which destroys the action of such a principle. If the former is correct the principle would differ from hormones like insulin which are known to pass through the placenta. It would likewise be different from many immune bodies circulating in the blood, since some of these are capable of passing through a placenta of the mouse type to the offspring. The evidence consequently leads to the conclusion that the material which the gene for this anemia has thrown out of balance is incapable of passing through the mother's placenta in amounts adequate for the offspring.

It seemed desirable, in spite of the negative character of the probable result from the use of materials which were capable of passage through membranes or of secretion in the milk, to attempt one other experiment using iron. For this purpose the salt ferric ammonium citrate was used. This material was fed to the mouse, injected into it, and fed to the mother before and after parturition. The material is toxic in large amounts but the animal appeared entirely normal if given a dose somewhat

under this toxic range. The result of these experiments on 12 mice gave no hope of success.

Whipple and his associates (5) have shown that by adding large amounts of liver to an animal's diet it is possible to alleviate both pernicious and secondary anemias. Three vigorous looking anemic mice from two litters were fed ground liver at birth; one was fed rat liver, the other two were fed mouse liver. They died in the usual time for anemic mice, 3 days, 4 days, and 6 days. Another set of mice gave like results. Liver seemed to be of no particular value to this inherited anemia. The liver concentrates might have been more beneficial. However, at this time we had found another method that was successful.

The injection of blood into the peritoneal cavity of the anemic mouse proved successful in maintaining life. There were, however, certain grave difficulties which had to be faced and overcome in accomplishing this purpose. The technical difficulties in obtaining and injecting blood into the mouse are such as to make it difficult to prevent the introduction of injurious contaminating organisms into the peritoneum of the young anemic mouse. And lastly, the abdomen of the anemic mouse is small, delicate, and filled with organs which are easily injured by the introduction of the injecting needle. Considering these difficulties, it is surprising that so many young have been favorably affected by the treatment.

Eighteen anemic mice were injected with blood from normal adult mice. In most cases the blood used for the initial injection came from one of the parents. Five hundredths of a cc was considered to be a large initial dose. The blood for later injections frequently came from unrelated mice. No obvious difference in the reaction occurred when this outside blood was used. Of the 18 mice treated, 11 had a duration of life well beyond that of the untreated anemics. Six had a life span nearly double that of the longest lived anemics. Three mice lived better than 2 months: one 67 days, another 85 days, and a

third 117 days. The average duration of life of 14 anemic untreated controls was $2.4 \pm .3$ days; that of the 19 treated anemics was 19.4 ± 4.9 days. The frequency distributions of the duration of life of the mice in the two groups is shown in Table IV. It is the extended distribution of life of the treated anemic mice as compared with their untreated controls rather than the average duration of life which is significant.

TABLE IV

DURATION OF LIFE OF THE UNTREATED ANEMIC MICE AND THEIR IMMEDIATE RELATIVES INJECTED WITH BLOOD OF THE NORMAL MOUSE

	Duration of life in days														Average
	1	2	3	4	5	6	7	8	12	13	14	67	85	117	
Untreated anemics	6	3	2	2				1							2.4 ± 0.3
Anemics injected with blood	3	1	1	2	1	1	3		1	2	1	1	1	1	19.4 ± 4.9

The physical development of the anemic mice that survived furnished new information on the action of the gene for this anemia when in the homozygous condition. On the whole, anemic mice at birth are somewhat smaller

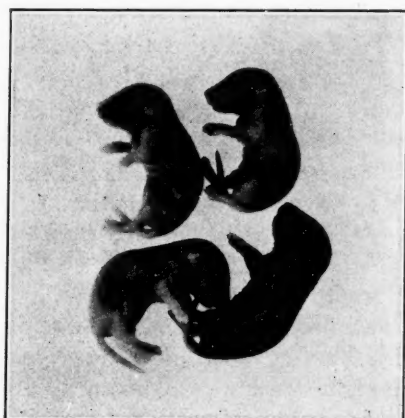


FIG. 1. Litter at birth, containing 3 anemics and 1 normal.

than are their litter mates. This may be seen in the photograph shown in Fig. 1. The degree of this difference is, according to our weights: anemics $0.97 \pm .03$ grams, litter mates $1.39 \pm .05$ grams. The anemic mice under normal conditions continually regress in size in spite of the fact that they suck large quantities of milk from their dams. The picture is clearly one of the mastery of the gene over the physiological utility of the nutrition. The normal litter mates grow rapidly from birth. When the anemics are injected with blood they commence to put on weight. They never completely overcome the handicap of reduced size however. Fig. 2

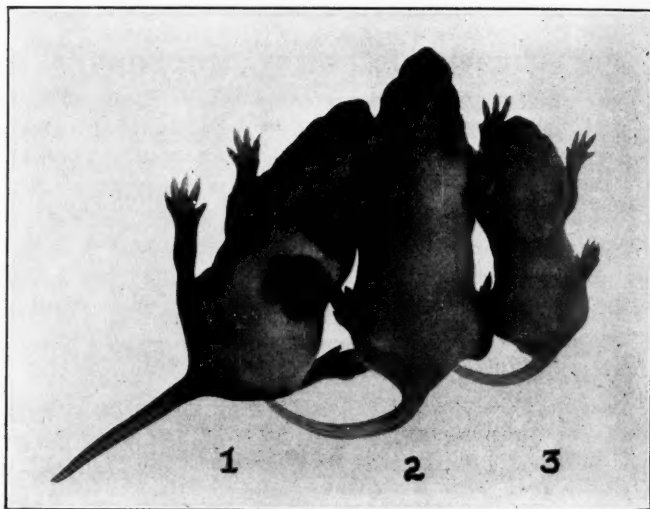


FIG. 2. Litter 7 days of age showing (1) a piebald, (2) a piebald with heterozygous dominant white spotting and (3) a piebald with homozygous dominant white spotting.

shows two normal litter mates and an anemic mouse which has been injected with blood. This anemic mouse had received a trace of blood the 1st day, and 0.05 cc on the 3rd. The photograph was taken on the 7th day. The anemic mouse is obviously smaller than the controls but at the same time, judged by the growth of the hair, is

nearly as far advanced physiologically. The anemic mice have never overcome the reduced initial size.

The anemic mice open their eyes about 2 days later than their litter mates. The three mice which lived to 67, 85, and 117 days were, respectively, female, male, and male in sex. An attempt to breed these animals together failed. The outcross in both directions likewise failed. Examination of the female showed that her vagina had opened at approximately the normal time, indicating the presence of estrin in the circulation. The animals were all small, delicate and underweight. The males showed but little external development of the testes. On the death of the female the ovaries were found to be very small, the size of those of an immature mouse. The testes of the males were also infantile in size. The histological study of the tissues will appear in a later paper.

The principle, which is altered by the gene for this anemia, may be replaced by the blood of the normal mouse when this blood is injected into the peritoneal cavity, but the anemic mouse is not able to initiate or continue its manufacture at a rate sufficient for the animal's needs and frequently repeated injections of blood are necessary to keep the animal alive.

The three animals which we were able to raise to the age of sexual maturity were inoculated every 2 days for the first week of life and every 3 days for the following week. The animals had then reached an age where it was evident that the type of treatment was able to bridge the pathology produced by the gene. The animals consequently became particularly valuable for possible breeding purposes, etc. Our experience had shown that each injection presented danger of infection or organ damage sufficient to cause death. For this reason the attempt was made to prolong the interval between injections as long as possible. The next injection was delayed for 8 days and the following one for 9 days without apparent ill effects. Nineteen days elapsed before the next injection, the animals being examined daily for symptoms of

insufficiency. The female of the group began to lose weight, her fur became rougher, and her movements sluggish. The animals were then injected, but while the two males showed their usual activity, the female's weight continued to diminish, her fur to roughen, and her actions to be increasingly sluggish. She died 4 days later. The males were injected May 16th and May 23rd at 8 and 7 day intervals following the death of the female. Just before the May 16th injection one of the males was bitten behind the ears by his normal female consort. He was separated from this female but on the 23rd of May had lost more weight and had become weaker. He died shortly after this injection. The last male was injected on May 23rd and 29th, and June 6th, and then no more injections were given for 18 days. By the end of this period his weight had decreased, his appearance was markedly anemic, his fur was rough and he was sluggish. He was then injected with 0.45 cc of blood. Although the animal lived until June 28th, or 4 days after this injection, his appearance remained unchanged.

These results point to the conclusion that after anemic mice reach the age of sexual maturity (we have normal black-eyed white mice bred 28 days from birth) these mice to survive must have a substitute for the principles affected by this hereditary factor. This substitute must be made every 2 weeks or oftener. The depletion of this principle brings the animal, after a time, to a threshold beyond which it can not be revived. It is an obvious suggestion from the character of the anemia that the vital materials affected by these genes and replaced by the blood are the red cells carrying hemoglobin. Other conceivable explanations are possible, however, and in any case we do not know whether it is the introduction of the red cells which act as carriers of hemoglobin or the formed hemoglobin itself which is responsible. Also, in view of the effects on the reproductive cells, it is clear that these genes manifest other reactions for which the introduction of blood does not compensate.

A similar case of replacement of the substance lacking through the developmental effect of a gene complex has been reported by Smith and Mac Dowell (6). Mac Dowell noted that in a silver strain of mice dwarfism appears in ratios suggesting a simple gene difference. This condition appeared suddenly when the mice were about two weeks old. The dwarfs were sterile and showed thyroid, adrenal and reproductive repressions suggesting pituitary insufficiency. Replacement of the deficiency by pituitary transplants resulted in resumed growth, normal thyroids and adrenals and reproduction. The anterior pituitary alone did not show repair. This case is even better than that here described in illustrating how the gene may bring about a deficiency causing a typical pathology which may be alleviated by replacement of the deficiency from the environment.

SUMMARY

The results herein presented show that this anemia may be counteracted by the injection of normal blood which replaces the vital principle or principles necessary to life not found in the presence of this gene. It is thus shown that the gene effect may be replaced by the substitutions of materials taken from without the animal.

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SHAPE CHANGES DURING FRUIT DEVELOPMENT IN CUCURBITA AND THEIR IMPORTANCE IN THE STUDY OF SHAPE INHERITANCE

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IN previous papers (1927, 1930) the writer has presented evidence that the inheritance of shape differences in the fruit of *Cucurbita pepo* is governed by a series of genetic factors differing in the character and intensity of their effect but clearly amenable to orthodox Mendelian analysis. He has also shown (1929) that the major shape differences visible in the mature fruit are evident in the very early developmental stages of the flower. Thus a typical "disk" fruit, which is much wider than long, will have a very similar shape index almost as soon as the ovary primordium is distinguishable and indeed when its bulk is no more than one millionth of that which it ultimately attains at maturity.

A more recent and intensive analysis of developmental changes in the fruit of *Cucurbita*, both in pure lines and in crosses, has abundantly confirmed these conclusions but has also established the fact that in the progress from ovary primordium to mature fruit there are certain minor but perfectly definite changes in shape. These changes are in many cases distinctive of particular lines, and are apparently inheritable. Thus there is a general tendency in most disk types for the ovary and fruit to become somewhat flatter as they develop, with a progressively greater width (equatorial diameter) in proportion to length (polar diameter). Such a change, for example, may be from an index of 1.5 W (one and one half times as wide as long) at anthesis to 2.0 W at maturity. Among the more nearly isodiametric types, this change is also evident but is less marked. In some types the

flattening is continuous from the first, while in others the young primordia at first become slightly more elongated until about the time of anthesis and then grow progressively flatter. Thus the curve of shape index plotted against volume of ovary (and fruit) has a somewhat different character in different pure lines, but in almost all disk and sphere types it rises appreciably between flowering time and the development of large mature fruits; that is, the index increases on the W side, or the structure becomes progressively more flattened, as it increases in size.

The size at which fruit maturity may be reached, however, is markedly variable in *Cucurbita pepo*. When environmental conditions are unfavorable, ovaries may develop into seed-bearing fruits when they have increased but little in size since anthesis; but under more favorable conditions they may enlarge remarkably, sometimes even to a point fifty times as great as the minimum size for maturity. It is significant that regardless of the size attained by a fruit when it stops growing and becomes mature, it retains essentially the shape index characteristic of that particular size on the shape-size developmental curve. Thus a group of plants belonging to a single pure line and essentially homozygous as to fruit shape but growing under diverse environmental conditions may produce mature fruits ranging in weight from one hundred grams to several kilograms, the smaller ones being relatively thick and the larger ones showing a slight but significant progressive increase in degree of flattening.

Furthermore, when two types differing in fruit size are crossed, there is a pronounced increase in the variability of this character in F_2 even though environmental conditions are very similar, presumably owing to the segregation of multiple factors for fruit size; and these genetic differences in size seem to affect the shape index in the same way as do the environmental ones, fruits which are genetically smaller tending to be somewhat

less flat than those which are genetically larger. Thus in a population free from segregation for shape there is a definite positive correlation between fruit size and degree of flattening. It should of course be understood that these genetic factors for size are quite different from, and independent of, the genetic factors for shape, as has been shown by the writer for this material (1931), and that the influence which size is found to exert upon shape is merely due to the fact that it modifies the phenotypic expression of a given shape genotype.

The importance of these facts for any study of the inheritance of fruit shape is obvious. In an F_2 population segregating for fruit shape factors, individual plants may differ considerably in the amount of soil nutrients available, in the incidence of fungous or insect attack, or in other respects which affect the vigor of the plant and thus the size to which its fruits will grow before ripening; and in many crosses involving fruit shape the parents also differ somewhat in fruit size, so that there are size differences in F_2 independent of the environment. All these size variations tend to modify the phenotypic expression of the genetic factors for shape and thus to blur the sharpness of their segregation and make genetic analysis much more difficult.

With a view to eliminating these difficulties, at least in part, an attempt was made during the past growing season to determine fundamental fruit shape differences in a few segregating populations by measuring the indices at a relatively early stage in development, when a given arbitrary size had been reached, rather than waiting for fruit maturity. Heretofore it has been customary to take the one or two biggest and best developed mature fruits on a plant (often there are but one or two fruits in all) and to record these as typical for the plant in question. In the case of three F_2 pedigrees, each resulting from a cross of a different disk line with a sphere line and showing clear monofactorial segregation, about 40 plants of each were grown. Of the total which

reached maturity, 92 proved to be disk fruited and 32 to be sphere fruited, a close approach to the theoretical expectation. Instead of determining the index only from a single mature fruit per plant, however, there were harvested and measured from each plant large numbers of ovary primordia and young and partially mature fruits, beginning with primordia only a few millimeters in diameter. For each of these the shape index was found and the volume computed. Enough determinations were made so that for every plant the characteristic shape indices of ovary or young fruit could be ascertained for any size or stage in development, and thus the whole segregating population could be compared, as to the shape index of its members, without the disturbing effect of size differences.

This major advantage could evidently be obtained by using determinations for any arbitrary size up to the maximum of the plant with the smallest fruits, but there were evidently two further advantages to be gained if relatively small ovary primordia rather than larger ovaries or fruits were chosen for shape analysis.

First, there is evident even in the same plant and at the same size and stage of development a certain amount of fluctuating variability of ovary shape, apparently due to differences in the conditions under which the primordium began its growth. These differences persist to later stages and make it advisable, even in the case of mature fruits, to average the shape indices of as large a number as possible. Such fruits are necessarily few on a single plant, but of young primordia the number available is very large since more are continually produced as earlier ones are harvested; and by the use of these larger numbers a more reliable and typical shape index for the plant can be determined.

Second, the earlier primordia are less affected by factors which control the course of later development. For each plant in these three pedigrees the curve of ovary and fruit shape index on volume could be rather accu-

rately determined, and a few typical curves for disk fruited F_2 plants are shown in Fig. 1. Some of these are

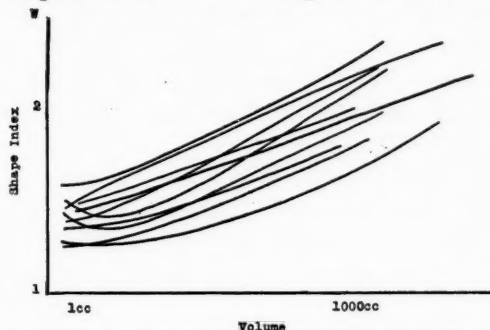


FIG. 1. Changes of shape index of ovary and fruit with increasing size, in ten representative F_2 disk-fruited plants of pedigree 631.

clearly steeper than others and the shape of the curves also varies, with the result that there is a considerably greater divergence in shape at a volume of 1,000 cc than at 1 cc. The cause of these differences probably lies in the segregation of minor genetic factors controlling growth and development, but it is evident that these exercise their influence relatively late.

To determine whether these presumptive advantages were actually existent, analyses were made of ovary shape in these three F_2 pedigrees at various stages. Data were especially abundant, naturally, for the small sizes and especially for the class with volumes between 1 and 8 cc (1^3 to 2^3). These primordia are about half the size of the ovary at anthesis. For each plant the shape indices of all primordia between the volumes of 1 and 8 cc were averaged and this value taken as the index for the plant. Then the index of the largest mature fruit (or the average for several, if available) was determined, as had ordinarily been the practice. These indices were plotted, the more elongate types to the left and the flatter types to the right of the isodiametric point.

The frequency polygons for these three segregating F_2 populations, both of the primordia and of the mature

fruits, are shown in Figs. 2, 3, and 4. In every case there is, of course, a considerable shift of the indices toward the right (flatter side) between primordia and mature fruits. The most notable feature of these figures, however, is the much sharper segregation and more symmetrical distribution of the indices of the primordia. In

F₂ 831

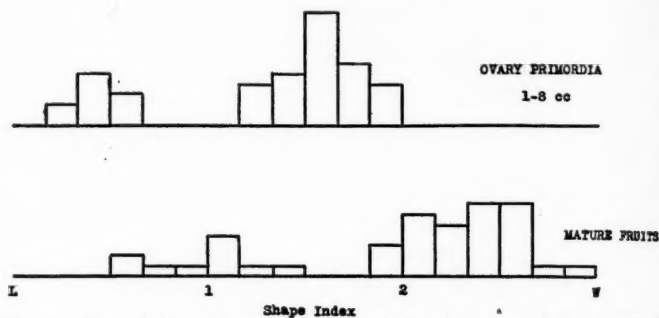


FIG. 2. Shape indices of ovary primordia 1-8 cc in volume (above) and of mature fruits (below) in F₂ 831.

F₂ 331

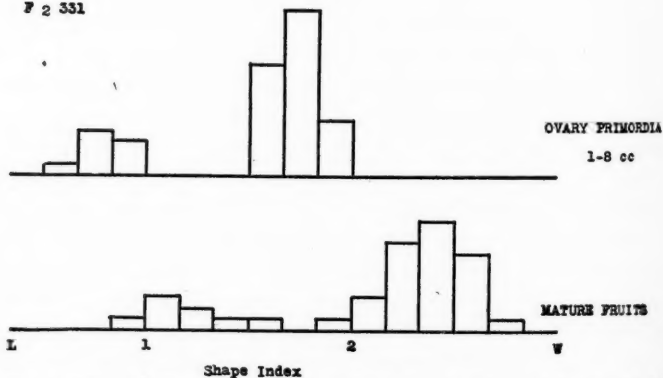


FIG. 3. Shape indices of ovary primordia 1-8 cc in volume (above) and of mature fruits (below) in F₂ 331.

F₂ 831, both primordia and mature fruits show clear segregation into two groups, but in the latter these are closer together and each covers a wider range. In F₂ 331, the two groups of primordia are also widely separated but

they approach closely in the mature fruits, on account of the increased spread of each. F_2 631 is particularly significant. Here the disk parent was much less flat than in the other two pedigrees and thus was closer in index to the sphere parent. Nevertheless, in the primordia the two segregating groups are clearly distinct, whereas in the mature fruits they have merged into a bimodal population. Here segregation is obviously taking place, but between the two modes occur individuals which on the basis of an analysis of the mature fruits alone could not be definitely assigned to either the disk or the sphere segregates. Knowing the shape of the primordia on

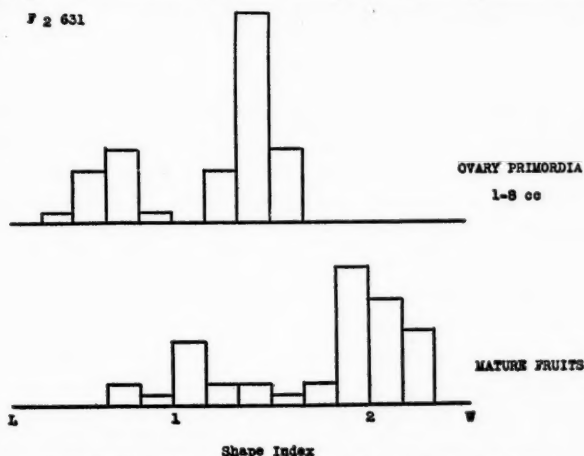


FIG. 4. Shape indices of ovary primordia 1-8 cc in volume (above) and of mature fruits (below) in F_2 631.

these plants, however, we could determine definitely which were genetically disk and which genetically sphere.

In these pedigrees segregation is obvious and genetic analysis easy from a study of the mature fruits alone, since the case is obviously a monofactorial one with dominance complete; but in more complex crosses, where the segregating groups are more difficult to separate, it is to be expected that a study of the ovary primordia will make the segregation more sharply visible and the

genetic analysis much more certain than as if mature fruits alone were investigated. Preliminary studies indicate that this new method will be fruitful in such complex populations.

The present study also indicates the existence, at least in this material, of two types of shape factors, perhaps not differing in kind but certainly in degree: major ones, which operate from the very beginning of development and almost from the start divide the plants into sharply different types, in the present case into disks and spheres; and minor ones, which exert their chief effect later, in modifying slightly the direction and rate of shape change during development.

SUMMARY

(1) During fruit development from early ovary primordium to maturity, in disk fruited and sphere fruited plants of *Cucurbita pepo*, there is a slight progressive increase in degree of flattening, or ratio of equatorial diameter to polar diameter.

(2) Fruits will stop growth and become mature at widely different sizes if environmental conditions vary or if there is segregation for size factors. The mature fruit retains the shape index characteristic of the developmental stage which it had attained when growth ceased. Thus the segregation of genetic factors controlling shape is rendered much less distinct if there are size differences in the population.

(3) By determining shape indices for small ovary primordia instead of for mature fruit, a population segregating for shape may be studied at a uniform size and the variability due to size differences may be eliminated. This method has the added advantages of making possible a much larger number of determinations for each plant, and of eliminating minor shape differences which often appear during development.

(4) In three F_2 pedigrees showing monofactorial segregation for disk and sphere fruit shape, a comparison

of the plotted indices of young primordia, 1 to 8 cc in volume, with those of mature fruits from the same plant showed in every case a much sharper segregation for shape in the primordia than in the fruits.

(5) It is believed that this method of studying earlier stages in development will make possible a more accurate analysis of shape inheritance in cases where the genetic situation is more complex.

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A STRUCTURAL CHANGE IN THE CHROMOSOMES OF MAIZE LEADING TO CHAIN FORMATION^{1, 2}

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AMONG the numerous strains of maize recently assembled for study at Madison one obtained from Manchuria through the U. S. Department of Agriculture was found to be segregating for partial sterility. Crosses between partially sterile plants of the Manchurian race, called M-sterile, and our standard, or o-normal line, give a certain proportion of incompletely fertile offspring. This behavior suggests that a structural change of a compensating character has occurred in the chromosomes of the foreign line giving rise to the M-sterile plants.

AMOUNT OF ABORTED POLLEN

Counts on 38 partially sterile segregates from the cross, normal ♀ × M-sterile ♂, show a mean percentage of defective pollen grains of 23.9. The variation in amount of abortion, however, is rather high, as indicated by a standard deviation of 4.83 per cent. One plant in the population produced 39.2 per cent. empty grains, and two plants gave a little less than 15 per cent. But aside from these three possibly aberrant cases, the variates appear to form a homogeneous group around the mean value. Slightly less than one quarter of the pollen grains formed by typical M-sterile plants are obviously non-functional. While counts have not been made, the partially filled condition of the ears borne by these indi-

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viduals indicates that a corresponding proportion of female gametophytes fails to develop. As will be discussed later, certain types of matings involving M-steriles give rise to individuals with much higher amounts of aborted pollen. These plants comprise a distinct class, however, and are termed "high steriles."

EVIDENCE FOR A SIMPLE TRANSLOCATION

Cytological examination of the pollen mother-cells of several M-steriles shows that at diakinesis they regularly form eight bivalents and a group of four chromosomes arranged in an open chain (Fig. 1). In normal maize,

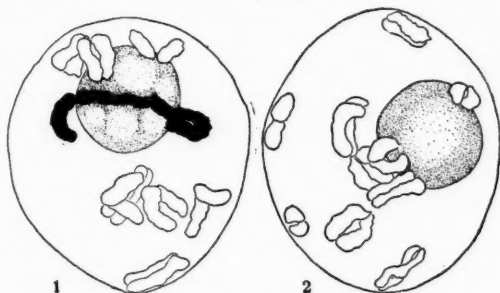


FIG. 1. Nucleus of microspore mother-cell of the M-sterile strain of corn at diakinesis showing 8 bivalents and a chain of four chromosomes.

FIG. 2. Same of normal maize showing ten bivalents.

on the other hand, ten bivalents are found at this stage (Fig. 2).

The occurrence of a chain of four chromosomes at diakinesis and the production of about 25 per cent. aborted pollen suggests that in the M-sterile race a simple translocation has taken place in which a terminal segment of one chromosome has been displaced and reattached by its broken end to the end of a non-homologous member of the complement. The postulated change in structure is illustrated diagrammatically in Fig. 3. If there is association of homologous ends at diakinesis it would be expected that the two modified chromosomes and their normal mates would be united in chain fashion.

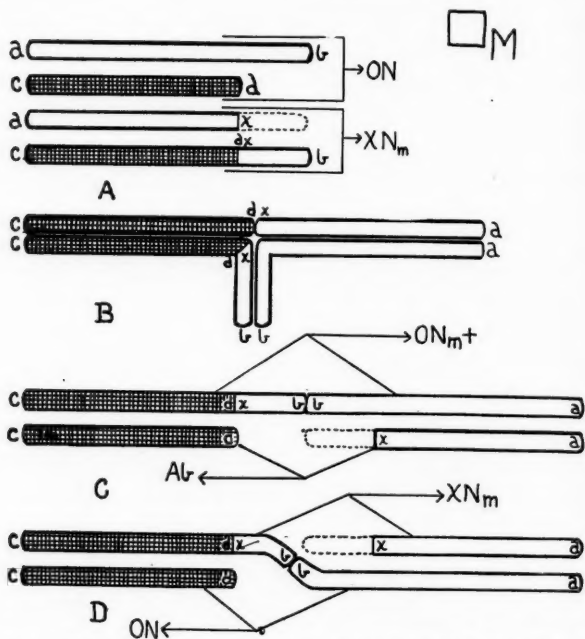


FIG. 3. Diagrammatic representation of the postulated change in structure bringing about the chain of four chromosomes in M-sterile plants. *A*. The two types of gametes; *B*. Type of figure to be found in the open spireme stage. Chains oriented on the spindle so that *C*, end chromosomes pass to the same pole and *D*, alternate chromosomes pass to the same pole.

If the group arranges itself on the heterotypic spindle in such a way that the deficient chromosome and its unaltered mate always pass to opposite poles and that the other pair assorts at random with reference to them four types of spores would be formed in equal numbers. As shown in Fig. 3, *D*, the one class of spores will receive the two unmodified chromosomes (o-normal); a second class will obtain the deficient chromosome together with the member of the other pair which has the translocated segment attached. These spores are potentially M-sterile producers and are termed x-normal. A third class of spores, as illustrated in Fig. 3, *C*, will receive a complement carrying the translocated piece in duplicate

($o-n+$); and the fourth type will be deficient for the displaced segment. It might be expected that the first three kinds of spores would be capable of developing gametophytes and that the last class would abort because it lacks entirely the genes carried by the translocated piece. On this hypothesis 25 per cent. aborted pollen is called for. It will be recalled that, on the average, 23.9 per cent. was found.

At metaphase in the pollen mother-cells of M-sterile plants two types of distribution of the chromosomes in the chain are commonly observed. In the first, as illustrated in Figs. 4 and 5, alternate chromosomes pass to



FIGS. 4 and 5. Equatorial plates wherein the alternate chromosomes of the chain pass to the same pole. FIG. 6. Equatorial plate showing the end chromosomes of the chain passing to the same pole.

the same pole. It will be seen from diagram *D* in Fig. 3 that this should result in $o-n$ and $x-n$ spores. In the second type of distribution, as shown in Fig. 6, the end members of the chain pass to one pole and the other two to the opposite pole. This mode of distribution presumably leads to the production of equal numbers of $o-n+$ spores and spores deficient for the translocated segment (Fig. 3, *C*). Since about 25 per cent. of the pollen is aborted, it may be assumed that the two types of orientation on the heterotypic spindle of the chromosomes in the chain occur with approximately equal frequency.

In 40 figures analyzed from four M-sterile plants, two cases were found in which the assortment was different from the above. The end members of the chain were evidently passing to opposite poles, and the other two

chromosomes were either failing to disjoin at all or were so oriented that if disjunction took place each daughter nucleus would receive two adjacent chromosomes. These types of distribution would lead to four aberrant kinds of spores, three of which would be deficient in chromatin material and would probably abort.

BREEDING BEHAVIOR

The breeding facts relating to the M-sterile race are summarized in Table 1. When simple chain-formers are self-pollinated about two thirds of the offspring are partially sterile and approximately one third are normal. Two families of this type were studied containing 35 and 39 plants, respectively. In the first group 68.5 per cent. of the plants were partially sterile and in the second 69.2 per cent. In matings of the type, M-sterile ♀ × normal ♂, a ratio of two partially steriles to one normal is also obtained. The one family examined gave 30 partially steriles and 16 normals. The deviation from a 2:1 ratio in this case is very small. On the other hand, the odds against the distribution being a chance deviation from a 1:1 ratio are about 32 to 1. The reciprocal cross, normal ♀ × M-sterile ♂, gives a different result. Eighteen families from matings of this kind showed 145 partially steriles and 154 normals, a relatively close approxi-

TABLE 1
THE BEHAVIOR OF THE M-STERILE COMPLEX IN INHERITANCE

Type of mating		Number of offspring	
		Partially sterile	Normal
M-sterile, selfed	Observed	51	23
	Expected	49	25
	(2:1)		
M-sterile, ♀ × Normal, ♂	Observed	30	16
	Expected	31	15
	(2:1)		
Normal, ♀ × M-sterile, ♂	Observed	145	154
	Expected	149.5	149.5
	(1:1)		

mation to a 1:1 ratio. The numbers in the breeding experiments are rather small, but the results appear to be of an orderly character.

Taken in conjunction with the cytological findings and the amounts of aborted pollen in the partially sterile segregates the breeding facts may be interpreted in the following way. M-sterile plants produce three equally frequent kinds of potentially functional spores, $o-n$, $x-n$ and $o-n+$. In the latter class the translocated segment is present in duplicate. All three types give rise to eggs. It is assumed, however, that the male gametophytes which are disomic for the translocated segment ($o-n+$) are markedly retarded in development and rarely participate in reproduction. Following self-pollination, accordingly, three kinds of eggs are fertilized by two kinds of sperms, as illustrated in Fig. 7.

Two kinds of normal zygotes will result, $o-n$ and $x-n$, through fertilization of eggs of these respective classes by sperms of like kind. These two types of plants are phenotypically indistinguishable from each other presumably, but are marked off from the other classes by the production of all sound pollen. Two of the six combinations shown in the diagram are the familiar chain-forming M-steriles. This class is easily recognized by its having about one quarter aborted pollen. Approximately half of the partially sterile offspring of selfed M-steriles, however, give amounts of aborted pollen significantly in excess of the 24 per cent. typical of chain-formers. Six plants of this sort upon which counts were made averaged 51 per cent. empty pollen grains. It was suspected that these "high steriles" represented the two classes of zygotes indicated in the right-hand column in Fig. 7. Such plants are hyperploids, being trisomic for the translocated segments.

HIGH STERILES

Cytological study of two high steriles has afforded evidence that this class of plants is actually trisomic for

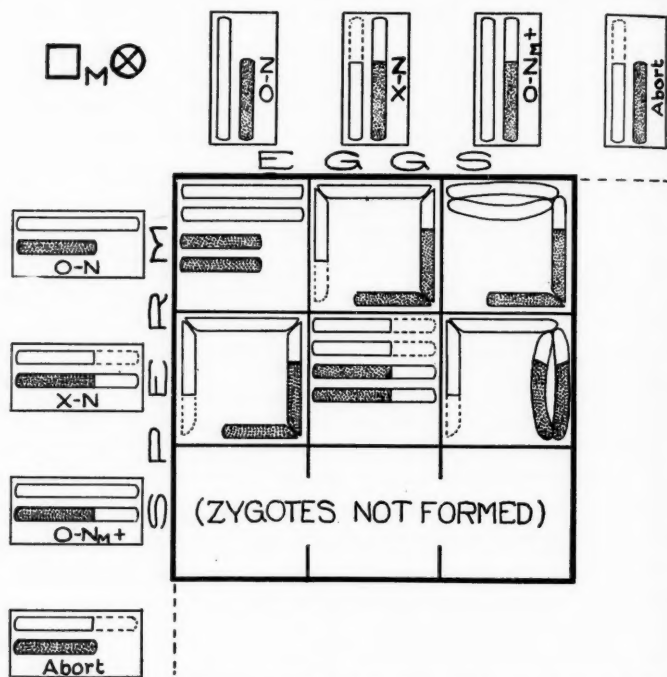


FIG. 7. Diagrammatic representation of the types of zygotes formed as the result of selfing an M-sterile plant.

the displaced chromosome piece. An early diakinesis stage in one of the high steriles is illustrated in Fig. 8. In the three-pronged chain complex, shown in solid black, two of the arms are clearly made up of two strands, whereas the third arm is composed of three. Fig. 9 shows the group separated into its component parts. Both members of the longer pair of chromosomes have a conspicuous lump near one end. The shorter pair consists of chromosomes unequal in length. This difference is due to the fact that the longer of the two chromosomes possesses the aforementioned segment containing the lump while the shorter member lacks it. Evidently the part showing the lump is the translocated piece and the plant is trisomic for it. In Fig. 10 a similar chromosome

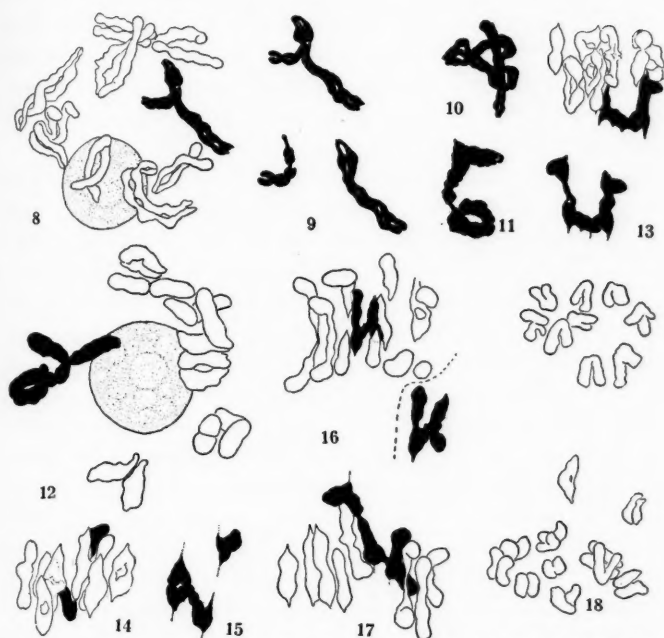


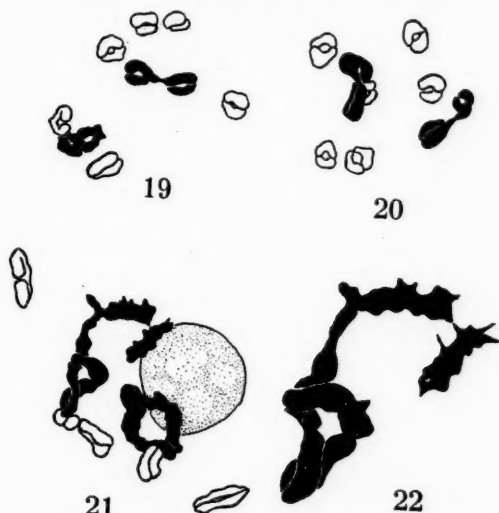
FIG. 8. Chromosomes of a pollen mother-cell of a high sterile plant at an early stage of diakinesis showing the chain complex and 8 bivalents. FIG. 9. The chain complex of figure 8 separated into its components parts. FIG. 10. A similar chain complex from another stage in diakinesis. FIGS. 11 and 12. Late diakinesis. FIGS. 13 to 17. The behavior of the chromosome complex on the heterotypic spindle. FIG. 18. Late anaphase showing 9 chromosomes at one pole and 11 at the other.

complex from the same plant is shown. In late diakinesis structures like those shown in Figs. 11 and 12 are found. In the latter figure it will be noted that the ends of three chromosomes are associated. In Figs. 13 to 17 the behavior of the chromosome complex on the heterotypic spindle is shown. Apparently non-disjunction occurs in a large proportion of the cases, since figures like number 18, showing nine chromosomes at one pole and 11 at the other, are abundantly found in the high steriles. The higher percentage of aborted pollen in this class of plants doubtless follows from the non-disjunction.

Hyperploids may arise from self-pollinated M-steriles in two ways: an $o-n +$ egg may be fertilized with an $o-n$ or an $x-n$ sperm. In their gross features the chromosome configurations resulting should be similar in the two cases (Fig. 7).

The amounts of aborted pollen in the partially sterile segregates from reciprocal crosses between normals and M-steriles should afford a test of the non-transmissibility, through the male gametophyte, of gametes carrying the translocated segment in duplicate. As stated in the second paragraph, only one plant in 38 partially steriles from the cross normal $\text{♀} \times \text{M-sterile } \text{♂}$ showed an amount of aborted pollen (38 per cent.) approaching that found in high steriles. In the case of the reciprocal cross, M-sterile $\text{♀} \times \text{normal } \text{♂}$, on the other hand, 14 of the 30 partially sterile segregates gave over 40 per cent. aborted pollen. Possibly $o-n +$ gametes do function occasionally through the pollen. The plant producing 38 per cent. aborted pollen mentioned above may have arisen in this way. Material was not available, however, from which the chromosome make-up of this individual could be observed.

Little can be said at present regarding which two of the ten pairs of chromosomes in maize are involved in the M-sterile translocation. It is expected that data from linkage tests will be available next summer. Cytological examination of hybrids between $x\text{-normal-1}$ and M-steriles show, however, that the semi-sterile-1 ring is independent of the M-sterile chain (Figs. 19 and 20). This rules out the *B-lg* and *P-br* chromosomes (Brink and Cooper, 1931). In hybrids with $xn_{1,2}$ (homozygous for the segmental interchanges found in both semi-steriles-1 and -2), on the other hand, the M-sterile chain is associated in a group of six chromosomes, an additional ring of four chromosomes being independent (Figs. 21 and 22). It appears, therefore, that the semi-sterile-2 ring and the M-sterile chain possess one pair of chromosomes in common. As Burnham (1930) has shown, one of the



FIGS. 19 and 20. Chromosomes of the hybrid x -normal-1 and M-sterile showing that the semi-sterile-1 ring is independent of the M-sterile chain. FIG. 21. Chromosomes of a hybrid between $xn_{1,2}$ and M-sterile showing 5 bivalents, a ring of four chromosomes and a complex involving six chromosomes. FIG. 22. The chromosome complex of six chromosomes. Much enlarged.

chromosomes involved in the semi-sterile-2 ring carries the *c-sh-wx* (aleurone color—shrunk endosperm—waxy endosperm) loci. Burnham also has unpublished evidence, which is cited with his approval, that japonica shows linkage with semi-sterile-2. The position of the japonica gene, however, has not been determined. It may lie within the tenth linkage group, which is not yet definitely established.

Through cytological examination of the high sterile segregates from the cross, M-sterile ♀ \times o-normal ♂, it should be possible to distinguish between the donor and the receiver chromosomes in the chain. Following this type of mating all the high steriles will be of the o-n + type, as illustrated in the upper right-hand cell in Fig. 7. The two identical terminal units in the group are normal representatives of the donor chromosome. Material of

this sort at a suitable stage for cytological study, however, is not now at hand, so that the question as to which is the donor chromosome must be left open for the present.

CRITICAL CYTOLOGICAL EVIDENCE FOR TRANSLOCATION

If the behavior of the M-sterile race is the result of the translocation of a terminal segment of one chromosome to the end of a non-homologue it should be possible to obtain critical cytological evidence of the structural change in the early prophase stages of the pollen mother-cell. If the translocated piece is attached in its new

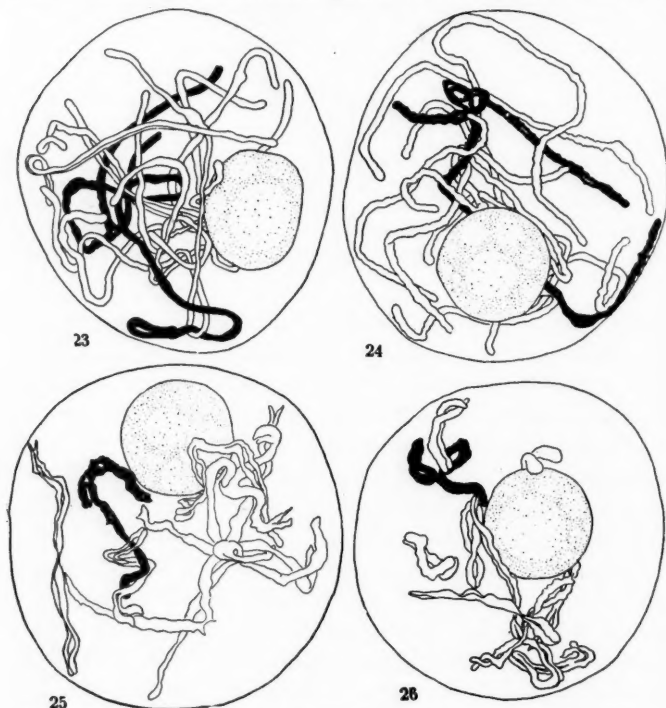


FIG. 23. Early spireme stage of a microspore mother-cell of an M-sterile plant showing a three-armed chromosome complex. FIG. 24. An open spireme stage. FIG. 25. A somewhat later stage. FIG. 26. Early diakinesis.

position by its broken end, and homologous parts of the two pairs of chromosomes synapse, a three-armed structure should result. A detailed study was made of the early stages of a typical M-sterile plant which produced 25.5 per cent. aborted pollen. Some of the configurations found are illustrated in Figs. 23-26. In the comparatively early spireme stages represented in Figs. 23 and 24 a three-armed chromosome complex, shown in solid black, is seen. Figs. 25 and 26 are based on older pollen mother-cells. Appreciable contraction has taken place, but the three-armed structure which will later form the chain is still evident. These cytological observations provide further crucial evidence that the M-sterile line is characterized by a simple translocation.

SUMMARY

(1) The M-sterile race of maize studied shows eight bivalents and a chain of four chromosomes at diakinesis. About 24 per cent. of the pollen grains abort and the ears are incompletely filled.

(2) M-steriles selfed give partially sterile and normal offspring in the ratio of 2:1. The cross M-sterile ♀ × normal ♂ gives a like result. The reciprocal combination, normal ♀ × M-sterile ♂, however, produces equal numbers of partially sterile and normal offspring.

(3) When M-steriles are used as pistillate parents about half the partially sterile offspring give amounts of aborted pollen considerably in excess of 24 per cent. These plants are termed "high steriles."

(4) It is assumed that in M-sterile plants a terminal segment of one chromosome has become detached from its normal position and affixed by its broken end to a non-homologous whole chromosome.

(5) The three-armed chromosome structure expected on this hypothesis in the heterotypic prophase has been identified and is illustrated.

(6) At the heterotypic metaphase, commonly, either the end members of the chromosome chain pass to one

pole and the other two to the opposite pole or alternate chromosomes go to the same pole.

(7) Of the four resulting types of spores one is deficient for the translocated segment and aborts, one carries a normal chromosome complement and one the two chromosomes modified by the translocation. The fourth type carries the "receiver" chromosome with the displaced piece attached and a normal "donor" chromosome; it is disomic for the translocated segment.

(8) The difference in reciprocal crosses is due to the non-transmission of gametes disomic for the translocated segment through the male gametophytes.

(9) Cytological evidence is presented showing that the "high steriles" are hyperploids. The trisomic condition of the translocated segment leads to frequent non-disjunction.

(10) The M-sterile chain possesses one pair of chromosomes in common with the semi-sterile-2 ring.

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METHODS FOR DISTINGUISHING BETWEEN DUPLICATIONS AND SPECIFIC SUPPRESSORS

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SEVERAL cases in which the principal effect was the suppression of a particular mutant character in *Drosophila melanogaster* have been reviewed or described in a preliminary way by one of us (Bridges; in press, *Quarterly Review of Biology*.) Some of these suppressions were clearly due to the presence of a duplicating fragment of chromosome whose suppressing action was through the wild-type allelomorph carried in the duplication. Others were attributed to the action of specific inhibitors which were mutations in a single gene different from and located far from the gene for the character suppressed. Some of the cases at first explained on the hypothesis of duplications have now been proved monogenic mutations instead. In this paper we wish to review briefly the older methods for determining whether a particular suppression belongs to the duplications or to the specific suppressor category, and to present a new method for distinguishing conclusively between these hypotheses.

That the suppressor is a translocated wild-type allelomorph rather than a mutation in another gene has been proved in certain cases by cytological demonstrations of the duplicating sections of chromosome. Even when the duplication is too small for cytological demonstration the genetic evidence may be strong enough to be conclusive. This was true for the suppressions of morula, speck, balloon and other mutants at the right end of the second chromosome through action of Pm-duplication (Bridges, '19). That the suppression is not due to a duplication of the wild-type allelomorph is clear when the suppression arises in a stock homozygous for the gene

suppressed. But even then the suppressor might still be due to duplication of the recessive mutant gene, with summation effects of the kind assumed in Stern's ('29) explanation of certain bobbed suppressions.

That a given case of suppression does not belong to the duplication category had been fairly certain in several instances, but had not been rigorously proved in any of them. In only two instances had a method capable of giving a decisive answer been applied, but in each case with inconclusive results.

The first of these instances was concerned with vermilion-suppression, which was at first interpreted as a duplication; in fact, the hypothesis of sectional-duplication was invented to account for the phenomena encountered there (Bridges, '19). Among other things, the absence of the expected diminution of crossing-over in the neighborhood of the suppressor and the fact that the suppression acted for vermilion and for sable, ten units apart, but did not act for the mutants miniature, dusky and furrowed between them, had raised serious doubts of the validity of the duplication hypothesis as an explanation of this case. A decisive test was sought in the interaction of "vermilion-deficiency" with the suppressor. If the vermilion-suppressor were really a duplicating wild-type allelomorph, then the combination $v/v\text{-def}/+^v$ should be roughly equivalent to the normal heterozygote, $v/+^v$, and hence be wild-type. But it was found (cultures 6927, 6945, Table VII, Bridges, '19b) that females carrying vermilion-deficiency in one X and in the other both vermilion and the suppressor, showed vermilion eye color and not the wild-type red of suppressed vermilion. Unfortunately for the certainty of the conclusion that the suppressor was not a duplicating wild-type allelomorph but was a recessive mutant with its proper locus near the left end of the X, was the doubt that the vermilion-deficiency was itself genuine. None of the diagnostic points of vermilion-deficiency were strictly unassailable, so that it remained only more highly proba-

ble that the vermilion suppressor was a recessive gene mutation rather than a duplication.

Bonnier ('27) suggested a test by means of the wild-type allelomorph present in the triploid or 3N type. He assumed that if the vermilion suppressor is a recessive gene near the left end of the X, two doses of this recessive should be ineffective in competition with a single wild-type allelomorph. This expectation was in conformity with the general rule, to which the anomalous behavior of plexus with Pm-duplication was the only striking exception. A vermilion triploid ($v/v/v$) carrying in addition two suppressors ($s^v/s^v/+^{sv}$) should not give suppressing action, and hence the eye color should be vermilion. But if the suppressor is a duplicating wild-type allelomorph, then the eye color should be red ($+^v v/+^v v/v$). Unfortunately, the single vermilion female which Bonnier secured and assumed to be of the required constitution turned out later to have carried only one representative of the suppressor instead of the necessary two. The vermilion eye-color of this 3N female was therefore not diagnostic. The red-eyed diploid offspring of this female were all equationals, and the percentage in which they occurred (7 per cent. of the diploid daughters) agrees well enough with the 11 per cent. found (Bridges and Anderson, '25) for genes at the left end of the X. Hence, although the triploid test suggested by Bonnier is theoretically sound, the nature of the vermilion suppressor was left still undecided.

For conclusive evidence we have made use of one of the duplicating fragments (Duplication 101) kindly supplied to us by Dr. Dobzhansky and obtained by him in x-ray experiments (unpublished). This duplication covers the loci yellow, scute and silver at the left end of the X and, at the right end, includes bobbed and the spindle-fiber. This fragment is cytologically demonstrable, and constitutes therefore a real duplication for the mutants suppressed. Males carrying the fragment in addition to one normal X are normal in fertility and in most of their somatic characteristics. Since the locus of the vermilion

suppressor is very close indeed to that of yellow there was a high probability that Duplication-101 would be found to include this locus also. Accordingly, females homozygous for vermillion and for the suppressor of vermillion were mated to males carrying the duplication.

TABLE 1
SUPPRESSED VERMILION ♀ [su-v v/su-v v] × Dup-101 ♂ [y sc/101]

March, 1931	Wild-type ♀	+ ♂ [su-v v]	v ♂ [su-v v/101]
3,189	81	43	44
3,190	57	14	44
18,879	97	37	55
Total	235	94	143

In the F_1 progeny (Table 1) all the sons carried vermillion and its suppressor, while approximately half of the sons got the duplication from their father in addition. As the table shows, about half of the sons were red-eyed, having the vermillion suppressed as usual. But the other half, carrying the duplication, showed vermillion eye color. That is, the duplication suppressed the suppressor. This shows that the duplication includes the locus of the suppressor and that the suppressor is a gene recessive to this wild-type allelomorph. If the suppressor had been a wild-type allelomorph of vermillion, then the presence of this duplication for the left end would have had no special effect upon the vermillion situation. The eye color would have remained red, *i.e.*, suppressed or "heterozygous" vermillion.

An even more striking demonstration was provided by the results of crossing these vermillion males, carrying vermillion and the suppressor in the X and also carrying the duplicating fragment, to females homozygous for vermillion and for the suppressor. The daughters (Table 2) of this cross were all homozygous for vermillion and for the suppressor, and half carried the duplication. The flies with the duplication were vermillion eyed, while those without it were the usual red (suppressed vermillion). In the vermillion-eyed daughters two doses of

the suppressor were themselves suppressed by one dose of the wild-type allelomorph carried in the duplication. The results of these two tests prove conclusively that the suppressor of vermilion is not a wild-type allelomorph of the vermilion gene but is a recessive mutant—a specific suppressor located at the left end of the X.

TABLE 2
SUPPRESSED VERMILION ♀ [su-v v/su-v v] × F₁ v ♂ [su-v v/Dup-101]

April, 1931	+ ♀ [su-v v/su-v v]	v ♀ [su-v v/su-v v/101]	+ ♂ [su-v v]	v ♂ [su-v v/101]
3,276	13	10	14	10
3,306	31	16	24	43
Total	44	26	38	53

Another of the early suppressors to which the duplication hypothesis was applied was that for sable. Since the locus of the sable suppressor is likewise at the extreme left end of the X, the same duplication, 101, should serve as a test here also. Females homozygous for sable and for the sable suppressor, phenotypically not-sable,

TABLE 3
SUPPRESSED SABLE ♀ [su-s s/su-s s] × Dup-101 ♂ [y sc/Dup-101]

March, 1931	Wild-type ♀	+ ♂ [su-s s]	s ♂ [su-s s/101]
3,216	15	9	6
18,878	12	4	3
3,191	115	30	21
3,193	51	13	13
3,194	64	16	20
18,875	22	13	7
18,876	91	34	39
18,877	89	34	28
Total	459	153	137

were crossed to males carrying Duplication-101. The sons (Table 3) which carried the duplication were sable, with spread wings; the sons carrying only sable and the suppressor were not-sable, and had the normal posture of wings. The spread wings are an accessory character-

istic of the sable mutant type. Thus, the presence of the wild-type allelomorph in the duplication dominated the suppressing action of the sable suppressor and allowed both the body color and the wing effects of sable to reappear.

Two separate stocks of suppressed sable were used in this test. The first was homozygous for the check character garnet (cultures 3,216 and 18,878), while the other was homozygous for the check characters tan, vermilion and garnet (remainder of Table 3). Both gave the same result with Duplication-101. It is thus clear that the sable suppressor is not a wild-type allelomorph of sable, *i.e.*, a duplication, but is a recessive specific suppressor of sable.

Since the interesting black suppressor described by Plough ('27) is located also in the extreme left end of the X, Duplication-101 would serve for a test of the allelomorphism of this suppressor. Plough had already come to the plausible conclusion that since a single dose of the suppressor sufficed in the male, while two were necessary in the female, a gene mutation and not a duplication was involved. Our experiments with a stock supplied by Plough demonstrate this conclusively. Males carrying Duplication-101 and heterozygous for black were mated to females homozygous for black and for the suppressor. The mothers were homozygous for the recessive characters purple eye-color and curved wings, while the black chromosome of the father was marked by the dominant Plum eye-color (Muller, '30). Those of the Plum sons (Table 4) that carried the duplication were black, as expected on the assumption of a gene mutation (*su-b/Dup-101*; *b/b Pm*). The other Plum sons, without the duplication, had wild-type body color, suppressed black.

Some of the black Plum sons, in which the black suppressor was dominated by its wild-type allelomorph in the deletion, were crossed to stock females homozygous for the black suppressor and for black purple curved. The offspring (Table 5) were all homozygous for black

TABLE 4
SUPPRESSED BLACK ♀ [su-b/su-b; b/b] × Dup-101 ♂ [+101; +/b Pm]

April, 1931	+ ♀	b Pm ♀	+ ♂	b Pm ♂	Pm ♂
	Mixed class	su-b/+ g b/b Pm	Mixed class	su-b/101 g b/b Pm	su-b g b/b Pm
3,310	72	49	38	19	23
3,314	55	38	33	15	12
3,316	29	27	22	14	16
Total	156	114	93	48	51

and for its suppressor, nevertheless that half which received Duplication-101 all showed black body color, since the wild-type allelomorph of the suppressor was introduced through duplication at the left end of the X.

In this case of black suppressor as well as the cases of vermilion and of sable suppressors, it is noteworthy that two doses of the suppressor are recessive to one wild-type allelomorph, thereby conforming to the general rule in triploid dominance.

These suppressors have been proved to be recessive mutant genes by the use of a duplication involving the locus of the suppressor. The technique involving use of a deficiency for the locus of the suppressed mutant could be applied to the purple suppressor found by Stern (*Z. f. Ind. Abst. u. Vererb.*) and by Bridges (in press, *Z. f. Ind. Abst. u. Vererb.*). These recessive suppressors, since they arose in a stock homozygous for the purple gene, might be interpreted as a small piece of chromosome II, containing the purple gene, attached to chromosome III. On this assumption four purple genes would be present in the suppressed purple flies, and the suppression would

TABLE 5
SUPPRESSED BLACK ♀ [su-b/su-b g b/b] × Dup-101 b Pm ♂ [su-b/101 g b/b Pm]

May, 1931	b Pm ♀ (101)	b Pm ♂ (101)	b ♀ (101)	b ♂ (101)	Pm ♀	Pm ♂	+ ♀	+ ♂
3,420	14	7	12	14	22	12	25	6
3,439	13	17	10	22	24	15	22	16
Total	27	24	22	36	46	27	47	22

be interpreted as the cumulative action on eye color of genes which in two or three doses could only make a purple color. The large secondary effects in the case of Stern's suppressor, including high inviability, complete sterility of both sexes, and strong reduction of crossing-over, served to make the duplication hypothesis still more plausible.

A viable deficiency for the purple locus was provided by the deficiency from Translocation-H (Morgan, Bridges, Schultz, '30), in which a small section taken from near the center of the second chromosome, and including the loci for hooked and purple, is attached to the Y chromosome. The H-deficiency can be detected by the small bristles and other structural peculiarities of the flies heterozygous for it. Thus it was a simple task to obtain flies homozygous for the purple suppressor carried by chromosome III, and carrying purple in one chromosome II, while the other chromosome II is deficient for the purple locus. If the suppressor is a duplication carrying the purple gene, in such individuals the number of purple genes is reduced by the deficiency to three, and hence the eye color should be again purple. The situation would be analogous to that in the purple-eyed fly homozygous for purple but heterozygous for the suppressor. If, on the other hand, the suppressor is not a purple gene but is a true third-chromosome gene having a recessive specific suppression effect in purple, then the deficiency for purple should not change the situation with respect to the suppression and the fly should remain wild-type, *i.e.*, suppressed purple.

In testing this hypothesis males were synthesized which carried black purple in one chromosome II. The other chromosome-II was Deficiency-H, while Duplication-H was carried by the Y-chromosome. One third chromosome carried the recessive purple-suppressor (Stern's) and the other the dominant marker Hairless. Such a male was mated to a female homozygous for black purple and likewise having Stern's purple suppressor in one chromosome III and Hairless in the other. In the off-

TABLE 6
 $(+/+, b\ pr/b\ pr, su\ (S)/H) \varnothing \times (+/Def-H, b\ pr/Def-H, su\ (S)/H) \delta$

Phenotype	b pr H \varnothing	b \varnothing	pr Def H \varnothing	Def \varnothing	H \varnothing	b H δ	H δ	b δ	+ δ
I	+/+	+/+	+/+	+/+	+/+	+/Dup	+/Dup	+/Dup	+/Dup
II	b pr/b pr	b pr/b pr	b pr/Def	b pr/Def	b pr/Def	b pr/b pr	b pr/Def	b pr/b pr	b pr/Def
III	su/H	su/su	su/H	su/su	su/H	su/H	su/H	su/su	su/su
3,076.....	31	7	4	1	1	29	23	2	16
3,108.....	45	17	11	1	34	62	23	29
3,210.....	20	3	1	16	15	1	8
3,215.....	44	3	1	24	30	2	6
3,217.....	24	3	1	13	16	2	10
Total.....	164	33	16	3	2	116	146	30	69

spring the deficiency appears in the daughters only. The daughters (Table 6) carrying Hairless were only heterozygous for the suppressor, but the not-Hairless daughters were homozygous for the suppressor. The daughters carrying the deficiency, easily recognized by their small bristles, etc., and homozygous for the suppressor, distinguished by being not-Hairless, in the only three individuals found, were wild-type in eye-color and not purple. Hence the suppressing action was not affected by the deficiency, and the suppression is accordingly not due to an additional purple gene but to a mutation of a third-chromosome gene. This is further evidenced by the lowered viability of the combination of Deficiency-H with the suppressor, which, were it a duplication for the region of purple, might be expected to neutralize the effects of the deficiency. Since neither in the somatic effects of Deficiency-H nor in its viability is there any neutralization, but instead a super-position of the phenotypic effects of the suppressor and the deficiency upon each other, on this count also, the suppressor must be considered as a mutant gene.

The test was extended to the purple suppressor found by Bridges (in press), which suppressor, while allelomorphous to Stern's suppressor, showed certain differences, including higher fertility. The compound between them has still higher viability and fertility, higher than either showed in homozygous forms. Females homozygous for purple and carrying suppressor-B in one third-chromosome with a dominant marker, Stubble, in the other, were crossed to the same type of male used above. The not-Stubble, not-Hairless offspring were suppressor (S)/suppressor (B) compounds. The two which carried deficiency-H (females only) were found to be wild-type in eye-color and not purple-eyed (Culture 3,298). Thus, the purple suppressor found by Bridges is likewise not a purple duplication but is a mutation of a gene normally present in the third chromosome.

The same type of experiment, differing principally in that the suppressor entered through the reciprocal parents, furnished the data of Table 7. Here again the

Phenotyp

Jan. 1931
Genotype

3,039

deficiency for purple did not undo the suppressing action of the third-chromosome mutant genes, as shown by the red-eye color of the seven Deficiency-bearing females.

The experiments reported in this paper have completed the proof that the suppressions in the cases discussed are due, not to section duplications, but to monogenic mutations in loci other than that of the gene suppressed. The several types of proof—through use of a deficiency, a duplication or a polyploid condition—are generally applicable, and, on the whole, of equal theoretical validity. As is obvious, certain loci can best be treated with a particular method, so that no extended discussion of the comparative merits of the different methods is called for. The triploids, 3N, generally present the greatest technical diffi-

TABLE 7

(+/, b pr/b pr, su(S)/H) ♀ × (+/Dup-H, pr/Def-H, su(B)/H) ♂

Phenotype	pr H ♀	+ ♀	pr Def H ♀	Def ♀	H ♂	+ ♂
I	+/+	+/+	+/+	+/+	+/Dup	+/Dup
II	b pr/pr	b pr/pr	b pr/Def	b pr/Def	b pr/pr or Def	b pr/pr or Def
III	su/H	su(S)/su(B)	su/H	su(S)/su(B)	su/H	su(S)/su(B)
3,039	41	18	15	7	68	42

culty, but on the other hand are not limited to those cases in which a duplication or deficiency has chanced to fall into our hands. By use of the x-ray method an increasing number of deficiencies and duplications are becoming available.

The bearing of these results upon Fisher's theory of evolution of dominance (Fisher, '30—a review) is obvious. The suppressors reported here are demonstrated to be mutant genes similar to those which Fisher has postulated to maintain the dominance of the normal allelomorph.

They differ in that they affect the phenotypic appearance of the homozygous recessives rather than the heterozygotes important in the consideration of dominance. But suppressors of dominant mutants in the heterozygous

condition are known, *e.g.*, the third-chromosome recessive suppressor of Hairy-wing. Yet, generally speaking, they are less fertile and viable than their sibs which do not carry the suppressors. Selection would, therefore, operate against most of them, as it does against the large majority of all new mutations, which indeed Wright ('29) has pointed out. There are, however (Bridges, '32, in press), some cases—*e.g.*, port suppressor—in which the suppressed form has been known to replace the original mutant type in a stock containing both. Such cases have been considered as supporting the theory offered by Fisher (1930, p. 55).

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RADIUM AND LETHAL MUTATIONS IN DROSOPHILA

FURTHER EVIDENCE OF THE PROPORTIONALITY RULE FROM A STUDY OF THE EFFECTS OF EQUIVALENT DOSES DIFFERENTLY APPLIED¹

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THE fact that irradiation of some species of living things induces gene mutations led to the suggestion that variability among organisms in nature might be due, in part, at least, to radiation from naturally-occurring radioactive substances, the implication being that such radiation might have played an important rôle in the evolution of species. Results subsequently published by several investigators have seemed to indicate that such might be the case.

Olson and Lewis ('28) were among the first to point out the desirability of testing experimentally the effects of natural radiation upon organisms. Babcock and Collins and the present writers, working independently, performed almost identical experiments to test this point. Using an electroscope, Babcock and Collins ('29) discovered a location in a street-car tunnel in San Francisco where the natural ionizing radiation was fully twice as great as the radiation in their laboratory in Berkeley. Accordingly their experiment was designed to compare the rates of occurrence of sex-linked mutations in *Drosophila* in the street-car tunnel and in the laboratory. Of 3,481 tests made in Berkeley 9 hatched no male flies, or a rate of 0.26 per cent., of lethal mutation. Two thousand five hundred tests made in the tunnel gave 13, or 0.52 per cent., of lethal mutation. While the difference

¹ The expenses in connection with the work reported in this paper were defrayed in part by a grant from the committee on the effects of radiation upon living organisms of the National Research Council.

in rate, 2.5 times the probable error, is not fully significant statistically, these authors considered that the results approached significance. Upon a reanalysis of the data showing the actual experimental variation in rate in the several subgroups in each of the two series, it appeared that the difference between the average rates for the two locations was increased: 0.275 ± 0.086 .

The present writers ('30) found that in an abandoned carnotite mine in the East Paradox Valley of Western Colorado the air was strongly ionized. In addition to the electroscope readings an attempt was made to compare the amount of natural ionization in this mine with that of one milligram of radium. Radiation in the mine was 0.39 times as intense as that from one milligram of radium when the rays were passed through a 0.156-inch lead filter. There were 2,860 test cultures, of which 7, or 0.245 per cent. ± 0.062 , produced no male flies. In the 1,308 control cultures one lethal mutation occurred, a percentage of 0.076 ± 0.051 . The difference between tests and controls is 0.169 ± 0.081 , a difference 2.09 times its probable error. The authors believed that, while this difference was theoretically not statistically significant, it might actually be so, and that had the flies been exposed for a much longer period, the results would have been more striking. In this connection it was suggested² that a series of equivalent amounts of radiation might be carried out in the laboratory and the time of exposure extended accordingly.

These two experiments, one in California and one in Colorado, although falling short of statistical significance, were consistent, nevertheless, in that both gave an actually higher rate of mutation in flies exposed to natural radiation than in the controls. Thus it seemed that these results strengthened definitely the plausibility of the suggestions that natural radiation might be responsible for the mutations which "are the grist of the natural selection mill with the resulting evolution of new forms."²

² Hanson and Heys, 1930.

Shortly after the publication of these results, however, Muller and Mott-Smith ('30) reported measurements which indicated that natural radioactivity is far "inadequate to explain the frequency of natural mutations." Calculations were made of the relation between the artificial and natural radiation effects in order to compare the induced and natural mutation frequencies. The measure of radiation intensity in both cases was the usual ionization per cc per second in air. Considering radiation from every possible source (outer environment, culture medium, the flies themselves, etc.), and taking the lowest average natural mutation value from observations by Muller, Altenberg, Babcock and Collins, Hanson and Heys, and others, Muller and Mott-Smith arrived at the following comparison:

- (a) natural mutation—without special treatment—1:150 of the highest value artificially induced;
- (b) ionization values— 7.2×10^{12} ions per cc for artificial treatment and 3.6×10^7 ions per cc for untreated material, or a ratio of 1:200,000.

These authors consider that the difference between the ratios, 1:150 and 1:200,000, is undoubtedly significant, particularly since for comparison they chose "the minimum possible value for the natural mutation frequency and the maximum value for the artificially induced mutation frequency, the maximum for the natural ionization and the minimum for the artificially induced ionization."³ This makes the natural mutation frequency "at least thirteen hundred times as high as it would be if it were caused solely by the radiation which the flies receive from their 'outer environment,'" and leads to the conclusion "that natural radioactivity is not the major cause of mutations, and of organic evolution, but that most mutations come about as a result of other causes."⁴

The validity of the foregoing conclusions was admitted by some only with great reluctance, and in this connection it was suggested that perhaps the small amount of

³ Muller and Mott-Smith, 1930, p. 279.

⁴ *Ibid.*, p. 283.

natural radiation had a disproportionately great effect owing to its being spread over so long a period of time.

Experiments by Hanson and his associates ('28, '29, '29, '31) both with radium and x-rays have shown that the induced mutation rate obtained always varied directly with the dosage as measured by the ionization in air, indicating a striking proportionality between the physical agent and the consequent biological effect. Oliver ('30), using five x-ray dosages, varying only in time factor, found the total number of lethals occurring directly proportional to the duration of the treatment. Demerec and Farrow ('30) have reported results indicating that at low dosages the increase in the percentage of primary non-disjunction is "almost proportional" to the x-ray dosage applied.

In spite of these findings, however, it seemed possible that the proportionality rule might hold over only a limited range of duration or dosage and not perhaps for durations of days or weeks at very low dosages.

PROCEDURE

Accordingly experiments were initiated with a view to determining within what limits the proportionality rule holds. A series of dosages as nearly exactly equivalent as possible were planned which were to be applied in different ways, that is, in one case high intensity for a brief period as compared with an equivalent dose of low intensity spread over a long period of time. The method of irradiation was such, it is believed, that gamma-radiation only reached the flies and all secondary beta-radiation was avoided. The alpha and beta-rays were excluded by means of a 0.5 mm platinum filter, which at the same time allows the passage of seventy-eight per cent. of the gamma-radiation. The possibility of secondary beta-radiation was eliminated further by filtration through a layer of specially prepared wax one centimeter in thickness, and by enclosing the flies for treatment in gelatin capsules. This supplementary filtration through wax

also serves to diffuse the rays giving even irradiation.⁵ In order to provide a food culture medium during the long treatments and yet avoid secondary radiation from glass containers, heavily paraffined paper boxes were employed as treatment chambers in place of gelatin capsules. Just how great a secondary radiation is set up by gamma-ray vibrations upon entering the body of the fly is a matter of speculation. Wild type males were used, as in previous experiments, and after exposure were mated to heterozygous bar-eyed females of the C1B stock.

An attempt was made to secure exactly equivalent dosages in two series as follows:

- I. 300 mgms radium, $\frac{1}{2}$ hour's exposure—6315.00 r-units.
 - 4 mgms radium, $37\frac{1}{2}$ hours' exposure—6315.75 r-units.
 - 2 mgms radium, 75 hours' exposure—6315.30 r-units.
- II. 300 mgms radium, 1 hour's exposure—12,630.00 r-units.
 - 4 mgms radium, 75 hours' exposure—12,631.50 r-units.
 - 2 mgms radium, 150 hours' exposure—12,627.00 r-units.

As a check on these, a heavy treatment of 4 milligrams for 150 hours (25,263.00r) was given. The distance in each treatment was the same, 10 centimeters. In order to be certain of equivalent doses in each case, ionization readings for the amounts of radium used were taken by means of an electroscope, and the total dosage translated into r-units according to the usual method: the reciprocal of the fall of the needle in seconds multiplied by the constant, K, gives r-units per minute. Since gamma-rays ionize a gas very slightly, radiation which is composed solely of gamma-rays can not be measured except by the secondary radiation it produces. Thus the calibrations given are for the amounts of radium used with filtration through 0.5 mm of platinum.

EFFECTS OF EQUIVALENT DOSAGES COMPARED

Upon scanning the sterility data given in Table 1, it is apparent that the percentages of sterility in both the

⁵ Watson and Lawrie, 1931.

parent and the F_1 generations are approximately directly proportional to the dosage, regardless of the mode of application. In the two series of equivalent dosages there is no difference among the resulting sterility values which approaches significance, the differences being several times less than their respective probable errors. These sterility values are absolute in that they represent complete sterility. In all matings where sterility was suspected a period of recovery was allowed, and any return to fertility noted. Males which showed apparently permanent sterility were remated to new females as a test. Complete permanent sterility is a new observation in this work. It is interesting to note that sterility in the F_1 generation follows the dosage closely, and that the F_1 sterility values in each instance are approximately one half those for the treated generation. Since the F_1 individuals are one generation removed from exposure, this result suggests perhaps the existence of some factor necessary for fertility which was affected by the treatment.

TABLE I
STERILITY DATA

Generation	Dosage in mgms Ra	Exposure time in hours	Total number of cultures	Number of sterile cultures	Percentage of sterility
$P_1 \times P_1$	300	$\frac{1}{2}$	187	8	4.2781 ± 0.9960
	4	$37\frac{1}{2}$	150	7	4.6666 ± 1.1616
	2	75	150	6	4.0000 ± 1.6000
	300	1	178	16	8.9887 ± 1.4451
	4	75	150	14	9.3333 ± 1.6018
	2	150	150	13	8.6666 ± 1.5499
	Heavy dosage	4	150	25	16.6666 ± 2.0528
$F_1 \times F_1$	300	$\frac{1}{2}$	650	13	2.0000 ± 0.3704
	4	$37\frac{1}{2}$	650	14	2.1538 ± 0.3841
	2	75	650	15	2.3076 ± 0.3892
	300	1	650	24	3.6923 ± 0.4988
	4	75	650	28	4.3077 ± 0.5371
	2	150	650	31	4.7692 ± 0.5637
	Heavy dosage	4	400	34	8.500 ± 0.9394

TABLE II
SHOWING THE LETHAL MUTATION RATES AND THE DOSAGES

Dosage in magnus Ra	Exposure time in hours	Ionization pro- portional to	Dosage in total r-units	Number fertile F ₂ cultures	Number lethal mutations	Percentage of lethal mutations
300	$\frac{1}{2}$	0.2105	6315.00	637	30	4.7095 ± 0.5661
4	$37\frac{1}{2}$	0.002807	6315.75	636	30	4.7169 ± 0.5670
2	75	0.001403	6315.30	626	29	4.5669 ± 0.5616
300	1	0.2105	12,630.00	626	61	9.7444 ± 0.7980
4	75	0.002807	12,631.50	622	60	9.6463 ± 0.7543
2	150	0.001403	12,627.00	619	59	9.5315 ± 0.7938
4	150	0.002807	25,263.00	366	74	20.2186 ± 0.4474

Table II shows the lethal mutation rates and the dosages in intensity and time and in r-units. The differences between the largest and the smallest mutation figures obtained in each series fall short of significance:

4.7169 ± 0.5670	9.7444 ± 0.7980
4.5669 ± 0.5616	9.5315 ± 0.7938
Difference 0.1500 ± 0.7980	Difference 0.2129 ± 1.1256

The heavy test treatment gave correspondingly higher sterility and lethal mutation rates. Fig. 1 shows these results graphically.

DISCUSSION

The above observations seem to indicate that even within these fairly wide limits the proportionality rule still holds, and the frequency of mutation produced corresponds with regularity to the energy of the dosage absorbed. Equivalent doses give as nearly approximately equivalent results as might well be expected in dealing with living things. The long continued release of electrons by a radiation source of low intensity produces sterility and mutation results equivalent to, but not greater than, a corresponding dose of high intensity and short duration.

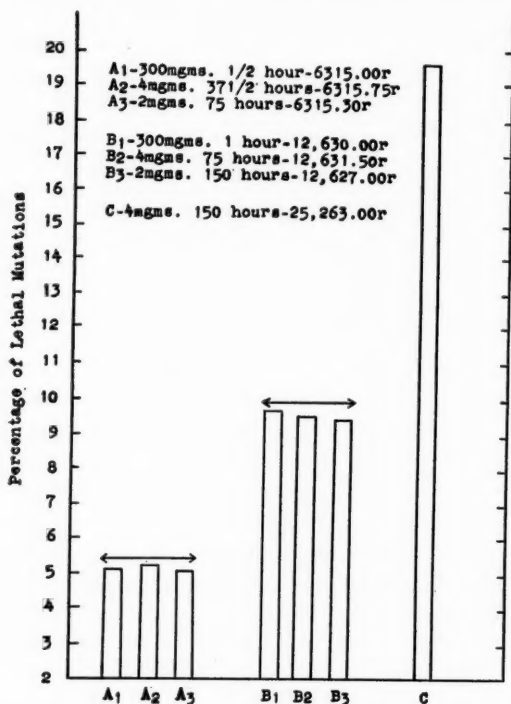


FIG. 1

Accordingly a small amount of radiation, such as that from naturally-occurring radioactive substances, does not seem to have a disproportionately great effect owing to its being spread over a long period of time. If, as the measurements and calculations of Muller and Mott-Smith show, the total radiation of the ordinary environment taken at its maximum is not adequate to explain the mutation frequency observed in the absence of treatment, and if, as the present observations indicate, dependence for induced mutation is strictly upon the total energy absorbed, regardless of the exposure period, we are still at a loss to account for the majority of natural mutations, and the question of the major cause of variation in organisms remains unanswered.

One can picture conceivably the occurrence in living tissues of a slow spontaneous process which might be accelerated slightly by natural and to a larger extent by artificial radiation. Studies of the differences in the susceptibility of cells under varying physiological conditions give evidence that the physico-chemical state of the cell at the time of irradiation influences radiation effectiveness. The authors are conducting at present experiments along this line.

When gamma-rays, which are of high vibration frequency and the quanta of which are, therefore, great in energy content, strike the atoms of cell substances, the transformed energy sets up both physical and chemical changes. The atom absorbs energy, has momentarily a greater "chemical sensitivity" or reactive ability, and can enter into combinations previously impossible. Whether chemical combinations already in progress are accelerated in the same direction or disturbed and sent off in another direction by this transformed energy is a matter of conjecture. However, Muller ('30) has pointed out from his own work on temperature and from a careful analysis of the results of Oliver with x-rays ('30) that applied radiation does not seem to act as a catalyst. It is conceivable also that the threshold of disproportionality has not been reached within even the very wide limits arbitrarily set in these experiments.

SUMMARY

Experiments planned with a view to determining within what limits the proportionality rule holds show again a strict correspondence existing between the amount of radium administered and the consequent biological effect, the induced mutation frequency obtained varying directly with the dosage.

A series of dosages as nearly exactly equivalent as possible but applied in different ways gave approximately equivalent results, dependence being merely upon the total energy absorbed. Whether the treatment con-

sists of a high intensity and a short exposure or low intensity and long exposure the dosage expressed in r-units is remarkably constant.

Within the limits set for the present investigation (2 mgms radium for 150 hours to 300 mgms radium for 1 hour) a small amount of radiation does not seem to have a disproportionately great effect owing to its being applied over a long period of time. Perhaps disproportionality begins outside even these wide limits, and, if so, there is still a possibility that a small amount of natural radiation acting over a very long time might be sufficiently effective to account for natural mutations.

The authors wish to acknowledge with appreciation the efforts of Professor E. Fauré-Fremiet, of the Collège de France, in securing laboratory space in which to carry on the experiments and to thank him for his constant interest in their progress. We take pleasure also in expressing our appreciation to Dr. A. Lacassagne of the Radium Institute, and to the Radiological Clinic of the Curie Foundation for arranging the radium treatments.

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THE STATUS OF THE SPECIES AND THE GENUS

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I. STRUCTURES AND ORIGIN

THERE seems to be no valid species, based on a single character. So-called physiological "species," differentiated by a single physiological character, are typical varieties, not subspecies, which are geographical units (see below). Any one species in a genus is differentiated from every other species by a *set* of morphological, as well also as physiological and habitudinal characters. Therefore a species originates, not by a change of one character, but by several contemporaneous changes.

Such combination changes are claimed to originate in three ways:

1. *By Hybridization.* This may occur where species overlap or where an individual is suddenly dropped into a new locality. If this field is fruitful, novelties should be sought for chiefly where congeneric species overlap.

2. *By Environmental Stimulus.* That there is such a thing has been amply proved by the North American Fauna series of the U. S. Biological Survey where one finds that desert regions consistently imprint certain characters on its mammals (also true of birds, snakes, insects and others), that humid areas consistently imprint their characters, dune areas theirs, etc., so that definite subspecies are found in each of these habitats with certain characters modified in the same way. These subspecific (geographical) characters have since been found to be constant on transplantation. Further, these changes check with "industrial melanism" in Lepidoptera (1), which phenomenon ought to be checked in the Pittsburgh region. Finally alpine dwarfing retains its characteristic in sea-level gardens.

Such environmental changes are known as mass changes, because they affect all the individuals of a popu-

lation subequally. I fail to find, however, a record of such subspecific (geographical race) variation being intense or extreme enough to be considered a full species. If, for instance, the geographical color change continued, the animal would eventually become albinistic—and albinism is not by itself a specific character, if it ever is. The same is true of melanism or any definite color. In other words *all* the colors of the species are equally affected. The pattern remains essentially the same, no new colors are added. Similarly, changes in size as dwarfing or its opposite are not the specific changes of changes in proportions. There are no combinations of structural changes in these subspecies. Thus geographical races are easily referable to their specific type. Such subspecies are, therefore, a standard systematic unit and throw no light on the origin of *species*.

(The same may be said of mutational varieties like albinism, xanthochroism, erythrochroism, etc., involving the loss of some *one* character or its appearance.)

The ornithologists—who have worked up their group more intensively than is the case with any other group, due in large measure to its huge corps of workers and its very early interest—have recognized these subspecies to the extent of ear-marking them as groups which freely interbreed. Objection has been raised to their stand (2, p. 54), on the basis that some western subspecies do not show interbreeding. Where this is true, it is for the very evident reason that whereas in the east, subspecies are limited by very gradual transition areas, in the west they are often separated by high mountain ridges, desert areas (affecting Cynipids), and other barriers so sharply segregating the subspecies as to make it impossible for them to interbreed. The ornithologist's ruling was merely intended to safeguard the species rank and was not intended as a definition of species and subspecies—hence is not comprehensive (inclusive of all subspecies). If the clause: "where interbreeding is geographically possible," were inserted, it would tend to forestall misunder-

standing. The fact remains that the ornithologists (as also the mammalogists) recognize the subspecies as definite geographical races of their species, freely interbreeding where the ranges overlap or meet (that is, on transitional belts). This is tantamount to saying that geography makes subspecies, but not species. A conchologist, who has spent many years in intensive study of fresh-water Mollusca of the United States, has found it necessary to adopt the same principle: "to consider a group of individuals which are separated from all other groups by some definite *combination* of characteristics, without intergrading, as species; those that show intergradations as varieties (3, ¶3).

3. *Mutations*. Our knowledge on this phenomenon is still so slight as to make it undesirable to attempt inferences. *Combinations* of specific characters, as is well known even to geneticists, are locked and can not yet be broken up by man. In *Drosophila*, for instance, by intensive inbreeding many aberrations have been produced, but none of the old specific character *combinations* (species) have been produced, no new ones made. The statement, "Since most of the mutants are *weaker* or less well adapted types than the wild type, they disappear before they are recognized" (4, p. 65), is undoubtedly intended more especially for bottle *Drosophila*. What do we know about mutations in the wild? What do we know about the contemporaneous mutation or recombination of *specific* (not aberrant) characters? Each genus, as it were, is a combination lock, and each of its species is a specific combination which is changed after a lapse of time. The species characters are impressed on each of several discs (chromosomes) and man has not yet learned to make up combinations (barring aberrations).

We might note in passing the effect of gregariousness and sedentariness as contrasted to the opposite habits on the rapidity of evolution. According to present concepts a recessive factor (incipient character) originating in an individual may be transmitted by breeding to many in-

dividuals, as a unit factor (possibility), but that it will not become evident (materialize) until two individuals bearing this recessive gene and of opposite sexes cross (4, pp. 59-65; 5, pp. 248-249). Thus a recessive factor which pops up in an individual of a species of animal having a tendency to live secluded in a clump of moss, or in a grass tussock or mat, will have far more of a chance of being disseminated throughout its companions and thus of becoming an actual character (by the union of that pair of recessive genes) than if it had occurred in a species having a tendency to climb trees every evening and then parachute off every morning. Likewise, such recessive characters would materialize much more quickly in a species of Mollusca hemmed in by high mountain walls or confined in an isolated group of trees than in a species ranging widely or promiscuously over a grassy plain. The tendency to live in herds or of returning after every migration to the same nesting site would also be advantageous to such rapid materialization of recessive factors. As a final example, a species living on mountain sides and having a tendency to ascend would become more and more concentrated at the top of the mountain because of its converging slopes. Thus mountain tops become centers for more rapid evolution. In brief, since inbreeding tends to bring out recessive mutations (not to mention dominant), evolution should be most rapid wherever the individuals of a species become concentrated, or lack the tendency to disperse.

II. STRUCTURES AND HABITS

In all three of these reputed originators students have been more concerned, like the systematist, with structure. "The Species Problem" (6) presents instance after example to show that there are exceptionally few real cases of correlation of structure with adaptation or selection and that in such few cases habit needs be also a correlative.

Habitat or any habit may be as much a specific character as the structural character, originating as an independent unit character, accompanying the morphological characters and subject to all the laws of specific unit characters in transmission and heredity (6; 5, p. 315 last word ff.). Because we can easily see, measure and describe the structural characters, we use them for species differentiation; the habits are lost in alcohol. It is the habits, however, that determine the numbers of a species in its food relations, protective relations, propagative relations. There is no better example of this than the study of the two British hares (6, pp. 197-202) in which the attempt is made to determine by a careful analysis of the specific differences in structure "how . . . structural differences . . . may be *explained by* the habits" for the supplanting of the one species by the other. The conclusions (p. 202) are that, "in Great Britain at least, the structural differences do not, with two possible exceptions [one *very* dubious], *correspond to* two radical differences in habit and mode of life in relation to which they have been of selective value." Following on, one finds the cue to the differentiation of habitat: "The Brown Hare has supplanted the Blue Hare very possibly on account of the boldness and more confident temperament [more inquisitive, on p. 198] of the latter which, as the country was brought into cultivation and more systematically hunted over, laid it open to destruction by men and dogs." This is a familiar story concerning several of our American wild animals. "Other examples (p. 209) are to be seen in the success of the Brown Rat (*E. norvegicus*) at the expense of the Black Rat . . . , of *Lepus americanus* at the expense of *L. arcticus* in Newfoundland" and others, or of unsuccessful attempts at introducing certain species.

After a careful review of all that has been done in the past eighty years to prove the value of interspecific structural adaptations in natural selection, the conclusion is reached (6, pp. 217-219) that we have no certain evi-

dence. If it did exist, after so much observation and experimentation, should it not be plainly evident? (Even the value of mimicry has been strongly questioned (7, p. 432). Finally all things failing, we are asked (6, p. 185, ¶1) not to be too rigid; not to insist on absolute correlation!

Again, a protistologist (8, p. 24, last ¶) found that in the dinophysoid flagellates, which are pelagic, there is no evidence of isolation and that "very closely related forms are not infrequently recorded in the same surface catch." By surface catch is meant uppermost fathom. "The ecologic niche which each [species] fills is in reality a wide shelf girdling the tropical seas and extending to a considerable depth. Moreover, many of these areas of distribution appear to be to a large degree coincident, even within the species" most closely related. This is undoubtedly true of most truly pelagic microscopic forms of life. Such results give us reason to doubt the efficacy of specific structures, or even habitats in determining survival.

Certainly it is not the structural, taxonomic ear-marks of the species that determine an animal's (or plant's) degree of adaptation to its environment or give it supremacy but its habits, more probably breeding habits. Natural (congeneric) selection is operative far more through habits (and habitat preference) than through superficial structure, and it is not until the morphological differences become so marked as to be of generic or family value (as reiterated by Robson) that they become of adaptational (selective) value and thus supersede the value of habits in this respect. Let us henceforth turn our eyes more and more, then, on habit studies as of *early* evolutionary significance, even though we must change from systematist to ecologist (sociologist).

Habitat restriction, by and large, becomes of no adaptive significance in the sense of a function of structural characters, but may be a specific character like color of nape, or spine of shoulder, or a habit acquired through

competition or restriction of range. Habitat preference in plants is in the nature of an adjustment (physiological) rather than an adaptation (structural). Were environment of such significance or so potent a factor, all species of an old Paleozoic or Mesozoic (unglaci-ated) area like some parts of China, would have the leaves of plants like Aster, Taraxacum, Erodium, Chenopodium, Viola, Astragalus, etc., growing side by side, all say strap shaped and hirsute; and the animals also would be reduced to the same superficial appearance through environmental convergence. The fact is, however, that other factors operate to keep species of unrelated genera and higher groups *different*, while the leaves, etc., of congeneric species are more similar than are those of unrelated species. It is only when the environment is so extreme (as in deserts, alpine regions, etc.) that it can counteract other influences enough to *visibly* impress itself and bring about structural convergence to any extent. In general, therefore, the physical environment is impotent in speciation, and any cases of special fitness of species in a not extreme environment would be exceptional rather than the rule. This seems also, by its negative results, to be the vintage of the years of research. Structural characters of specific rank are facilities to the systematist but only rarely of immediate value to the species in its livability. Our point of view has been extremely anthropocentric. "The Species Problem" is a monument to the lack of adaptational value of specific structural characters, and coming from an English biologist is of double moment.

III. GENERIC DEVELOPMENT

On the above basis, a species may be described as life forms related to each other, by the possession of the same combination of structural characters which are usually so poorly developed as to be of no immediate adaptive or selective value in the process of evolution, as well as by the possession of a combination of similar physiologi-

cal and habitudinal characters. The structural characters are so small or poorly developed that the environment has no effect on them. These various specific characters continue being modified (from within) and recombined (within the genus) to form, through time, more and more species until the generic area becomes so crowded as to bring about congeneric relations. Thus congeneric competition develops through such non-morphologic characters as "temperament": aggressiveness, recessiveness, inquisitiveness, audacity, pugnacity, secretiveness; vitality statistics (birth rate, fecundity, number of broods, care of young, etc.) and other sociological relations and habits. As examples of the intrageneric fitness (not necessarily competition) see 6, p. 209. Thus by intrageneric (congeneric) fitness or superfitness (not structural adaptations) the "weaker" species are crowded out, or more and more restricted in their distribution or niche while the more successfully prolific, the more viable, extend their area until the genus has many species throughout the zone or globe, some restricted, some more extended and one holotropic, holarctic or circumpolar. From this time on, generic senility occurs—due largely to the competition between the various congeneric species and the wide. Finally one of two things may occur: (1) the wide may become the single representative of the genus—as in *Nyctea* (snowy owl), *Scotiapteryx* (great gray owl), *Surnia* (hawk owl), *Cochlicopa* (Mollusca) and many invertebrates, or it may occur with a few species (as often), (2) the wide itself may drop out leaving a few widely separated (relict) species—as in *Bombina* (Amphibia).

To summarize: A sedentary or gregarious "parental" form (phylogenic genotype) throws off various combinations of characters, each at a different center, each of which, by the growth of reproduction, advances from its center like an eddy across the country, influenced in its spread chiefly by "barriers." Each offshoot begins from the parent at a different time and place and is held

constant by heredity. The eddies of range extension may be in any direction or have a general trend, depending on local factors governing distribution of species. At any time, however, some of these radiating species may in the course of migration meet a confrère with which it is capable of interbreeding and thus in turn become the center of origin of a radically new type as a subgenus or genus. Such may be illustrated by *Artemisia* (9, pp. 34, 49), *Cynips* (Hymenoptera, 2), *Thamnophis* (Ophidia, 10) and others. If there is any truth in emergent evolution, as there undoubtedly is, these crossbreeds would produce something new and would not necessarily show combinations of the old elements (parent stocks). If this interpretation be correct, phylogenists should seek the parental species among the most sedentary or gregarious of the group.

As emphasized in "The Species Problem," it is only when structural differences are of generic, more especially of family rank that they are positive enough to be of *selective* value. This should be paramount to saying that families are of greater evolutionary significance than species and therefore more rapid in their development and senescence. Taxonomically, the genus is the problem.

IV. THE QUESTION OF SURVIVAL

Darwin, in order to bolster up the survival of the fittest end of his theory, very much overemphasized mortality statistics. More careful studies of living forms have shown that the eliminated are to a large extent the young, the old and the diseased. In the case of some species (salmon) the death rate among the old reaches nearly as high as 100 per cent. per annum. Provided that they are not eliminated before spawning, their elimination is of no consequence to the species. In other cases, say birds, it is usually the older that are eliminated, or, according to Howard (11), the males when too numerous.

According to recent work in ecology, a climax formation, as a beech or spruce woodland, is a balanced as well as a closed association. In such an environment, a new species would have more chance for survival from predators than the old because the new would be correspondingly more rare (say one or more couples among tens of thousands of the old). And it is not until its numbers would be equal to those of the old species that it would become equally eliminated (other things being equal—and structural congeneric characters almost always are).

For example: Suppose the new species be a brown-winged warbler with one white cross-bar, a black line over the eye and a white bib, otherwise like the blue-winged warbler of the northeastern United States. Such a combination of color is no more conspicuous than any of the other warblers. Its size may be a few millimeters (a quarter of an inch) longer or shorter than the blue-winged warbler, the relative length of tail, wings, bill, may also be a few millimeters different. All these differences are of no particular advantage. Its numbers are two, a male and a female (one could begin with less). Would its chances of survival be less than those of any other warbler? If its disposition were such as to perch on the end of exposed twigs to sleep, it would undoubtedly be eliminated in short order. If its psychic habits were about the same as other warblers, it would not be wiped out any earlier than the neighboring pair of a related species. If it is associated with nine pairs of blue-winged warblers in a wood, it would stand the chances of one in ten of being killed by warbler enemies (high mortality being during youth, including eggs, or old age). If it were more secretive in disposition its chances of survival would be greater, if less secretive less. There would be no elimination by mating rivals because there would be no male competition, as males of another species would not recognize it as a rival. If its egg complement and care of young were similar to that of the blue-winged warbler it would increase normally

until as numerous as the blue-winged warbler, provided that the woodland's capacity for it were also nine pairs. The number would then remain at nine, neighboring woodlands receiving the surplus (or squirrels, snakes, etc.). If the egg complement were greater, it would increase to woodland capacity more rapidly. If each male were satisfied with a smaller territory than the blue-winged warbler that woodland would contain a greater number of pairs at optimum increase. The question of available food is one which varies with insect population, from year to year, and ability to vary its food habits. Degree of security from enemies varies with density. For instance a snake or squirrel would have to scrutinize fewer bushes or trees to find a nest of young if there are thirty nests in a wood than if there are nine. Also the more frequently the snake feeds on one kind of animal, the greater are its chances of getting heavily parasitized and thus weakened. This ratio between density of prey and degree of parasitization may account for the relatively low numbers of predators. As soon as prey fall below a certain number, the normal predator meets with other food while seeking for the few of its normal diet. Thus a balance is kept, not so much by habits as by ratio of size of prey relative to size of its habitat or cover, relative to the covering ability of its predator. Numbers in excess of this optimo-minimum is of no advantage to itself unless the prey harbors a parasite of the predator.

Exactly the same conditions would obtain for herbivores except that their food supply is less precarious. Furthermore, the smaller the life form, the less chance for elimination because of additional ease of secretiveness and the much greater distances that must be covered by the predator.

Thus it may be seen that the real question of survival of new species is not dependent upon small morphological characteristics (always with rare exceptions) but first, one of propinquity (both temporal and spacial) of one

male and one female and second, temperament, disposition, habits, especially breeding habits, and such characteristics.

As to food "competition" with the old species, fluctuations in numbers (12, 13), which is probably less frequent in climax associations, would bring in a chance factor to the extent that when the species is numerous in individuals, it is more quickly consumed, a rare remnant almost always being left. Near exhaustion of food supply often leads to migration of the predator. Different conditions would occur in different groups of plants and animals. Generalities of this sort are discussions in vacuo. The field of sociology is to secure the data for each species under its various conditions. My experience in unbalanced (non-climax) woodland is that there is a great deal of "room" to spare, either for sunlight or for absorption; either for nests or for food. As a single factor, consider the number of leaves that fall in the autumn, uneaten. In north China, the pagoda tree (*Sophora japonica*) is entirely defoliated in June by the Geometrid moth *Macaria cinerearia* (14) and immediately puts out a second crop of leaves. This is also the case with *Ulmus pumila* in late August, new leaves coming on even so late in the season. But out of fifty neighboring trees of *S. japonica* on the university campus, only two were completely defoliated. Some were barely touched. Two months later they were completely refoliated. Any one seeking for parasites knows that usually only a small percentage of hosts are infected (15, ¶ 1). In a higher vertebrate, of forty-three parts liable to infection by more than that number of species of parasites, one usually finds a total of four or five species of non-protozoan parasites per individual. Many kinds can be harbored if the number of individuals of each kind are few. Of leaf miners only an occasional leaf is affected (with rare exceptions). What birds have developed the habit of feeding on leaf miner larvae? The great bulk of forest leaves are unmined. Other ex-

amples are numerous, there is ample food for every one as long as numbers do not become excessive—and this usually occurs only on the heels of man. Even in semiarid north China, quantities of grass on the hills are left uneaten and are broken to dust by winter time and swirled into high heaven by the fierce wind storms of spring. This problem of supply and numbers will be equated eventually by the sociologist (certain ecologists).

Finally we are reduced to a consideration of birth rate and here if anywhere is the crux of the matter. Some advantages may be secured by the new species:

- (1) In increase in number of eggs and/or number of broods.
- (2) In increase in fertility (per cent. of eggs hatching)
- (3) In increase of care of nest (decoy of eggs)
(with or without increase in number)
- (4) In increase in care of young.

The first may be secured by increase in size of adult or decrease in size of eggs. The fourth may be secured by increase in size of eggs with further internal development of young. The *possibilities are numerous*. Any change here gives the species a real interspecific advantage or disadvantage.

There is no better example of this than among the Anura of southern New England. In this restricted area there are ten species. Among eight of these species there is very close similarity and in some cases a pair are separable only by close observation. These couples are *Bufo lentiginosus* and *B. fowleri*, *Hyla pickeringi* and *H. versicolor*, *Rana pipiens* and *R. palustris*, *Rana clamitans* and *R. catesbiana*. As already pointed out (16, p. 25), Europe has but one species for each of these pairs; a similar condition obtains in north China. Thus it seems that northeastern America has nearly twice as many Anurans as are to be found as a general thing in the transitional zone. This condition should bring on "competition" of an acute kind—if such exists. To my knowledge, no one has yet pointed out how the structural

differences between the two species of toads give the one an advantage over the other, nor how the specific differences between the four species of non-sylvan frogs (we have also a woodland species) give the one an advantage over another.

In the case of the toads, the only tangible differences are that *B. fowleri* breeds later (the breeding habitats are identical), and that it is more confined to sandy and drier localities (17, 18). This segregates *B. fowleri* to the sandy coast and river regions(18). Although there is overlapping of ranges the two rarely breed in the same ponds, neighboring ponds usually being occupied by one species or the other. "Competition," if it exists, should be easily observable and measurable in the pond areas where both species are found. When is or was this segregation established? Is psammophily due to a structural character (they barely differ structurally) or to a hormone, or is it "merely" habitual?

In the case of the *Hylas* the following biotic conditions exist:

	<i>H. pickeringi</i> (crucifer)	<i>H. versicolor</i>
Size	About 1 in.	2 in.
Size of habitus	Bushes, fences	On trees
Spring appearance	March 26	April 28 (at Boston)
Egg complement	1,000 (singly)	2,000 (small bunches)
Buoyancy	Demersal	Floating
Jelly envelopes	One	Two
Larval period	75-90 days	40-60 days
Size at transformation	Smaller	Larger

From this one would infer that *H. versicolor* would have several decided advantages. Yet it is recorded at Northampton, Massachusetts, as not rare (19) and *H. crucifer* as common. At Ithaca, New York, *H. versicolor* is less common (16, p. 2). On the whole there is no interference in breeding habits and probably not in niche occupied, but the latter should be carefully looked into.

The data for the four frogs follows:

	<i>R. pipiens</i>	<i>R. palustris</i>	<i>R. clamitans</i>	<i>R. catesbiana</i>
Size	3½ in.	2-3½ in.	3-5 in.	7-8 in.
Habitus	Meadows	Cooler waters	Meadows	Deeper water
Spring appear- ance	March 28	April 3	April 7	May 20
Egg complement.....	4,500	3,000	4,000	20,000
Buoyancy	Demersal	Demersal	Floating	Floating
Jelly envelopes	Two	Two	Two	One
Larval period	60-80 days	75-90 days	1 year	2 years
Size at trans- formation	Same	Same	Larger	Largest
Relative numbers ..	Most ab.	Less ab.	Solitary	Least common

As to habitus they all overlap and may all be found in the same pond. In fact one feels that their outstanding student (16) labors extremely to find a habitat difference. It can not be said that it is clear cut and definite. The egg buoyancy is correlated with season of appearance, namely after the last ice or frost, and secondarily, they get the full heat of the sun.

The most striking phenomena are: that the largest has by far the most eggs but is least common—undoubtedly due to its long larval period (a primitive condition—as well as the single jelly coat). Thus *R. catesbiana*, our largest frog, may be considered to be on the decline. By contrast, the most common species lays a fairly large egg complement, appears earlier than most of its enemies (water snakes, turtles, herons, etc.), has the shortest larval period and thus becomes amphibious and saltatorial earlier than any of the others. Here then are cardinal biotic factors which are actually equivalent to greater survival value.

In connection with the above, notice should be taken of the habitat "preference," namely that the big species take to big wood or big water, relegating the small species to what is left. Is this size sorting due to "combat" or is it merely mechanical, the mesh of the habitat acting as a sieve? Studies carried on in this region on *Rana* and *Hyla* should throw light on this problem.

Similarly, is *R. palustris* relegated to cooler waters by *R. pipiens* or does it take to cooler water through physiologic urge? Is its longer larval period due to cooler waters or is it so long irrespective of water temperature?

Vitality statistics (under natural conditions) on the eggs (aeration, per cent. fertilized, etc.) might throw more light on relative numbers. That feeding idiosyncrasies affect health has already been pointed out (20, p. 206) especially in the case of one individual whose lung cavities were almost filled by parasites, while others were comparatively free. If such habits become general in one species it would become markedly reduced in numbers.

Under this section one might also note, in the dino-flagellates (8, p. 24) "that the most highly specialized genera . . . and the most highly specialized species . . . are, in the main, relatively rare in individuals. The development of adaptive structures of a highly specialized type is not in many instances accompanied by a corresponding reproductive vigor or equal enlargement of survival value."

V. ORTHOGENESIS

I have recently reviewed a monograph (21) on a group of protista in which the writer repeatedly discusses orthogenesis within each genus, arranging his species according to this orthogenetic development or that. In a similar way I may arrange all the different types of bottles found in a Chinese junk shop in orthogenetic series—basing the series on size, degree of coloration, surface sculpture, or, were I a limnologist, I might consider them as single celled organisms falling into two series: (1) those with large bodies, (2) those with long necks. The orthogenetic climax of the first group would be those with the smallest neck and roundest bodies (greatest capacity versus surface), while in the second those with the longest, slenderest necks would be the climax forms as supplying greater floatability through

development of a spine-like process, or better controlling orientation in the medium, or anything else my imagination could devise that seems probable. Orthogenesis within the genus is purely imaginary, for biotic orthogenesis requires a time interval equivalent to family or even ordinal development to become established. If the elephant's trunk is an example of orthogenesis, one would not refer to the relative differences in length of trunk within the genus *Elephas* or *Mastodon* as even indicative of orthogenesis. It is by comparison with other genera or subfamilies of the Ungulates that one gets the perspective necessary to appreciate orthogenesis. The genus, as far as structure alone is concerned, is merely a group of combinations of similarly non-adaptive structures, a pushing out in all possible directions. No highway has yet been discovered or determined, much less established. It is not until these structures have been developed so far as to be of adaptive significance that they can be regarded as orthogenetic. The genus is heterogenetic. The proof of this statement is that in the above mentioned monograph (21), as in other similar systematic contributions, the genera are divided into subgenera, each of which has its own line of development. In some cases the number of species in the subgroup is as low as two with one more "primitive" than the other in one *character*! Worse yet, in at least one such genus a special group of heterogeneous forms consisting of extremely simple and extremely complex structures, and not fitting into the other subgroups, is made! In other words even this clever "orthogeneticist" finds numerous exceptions to his devious lines. Orthogenesis postulates one line only through an *order*, various structures being in harmony. Why not forego such an orthogenetic urge and regard the genus as an astral pattern?

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The genus thus becomes a producer and testing plant for its own produce, for the most "efficient" species (one or more) having certain specified (generic) char-

acters—all the producing and all the testing being congeneric, a self-sufficient plant. Within wide limits, the environment is not functional; these limits being so wide as to allow for a great deal of specific latitude and congeneric interplay.

To what extent these tested species interplay with the tested species of other genera of the same family or other families depends on how similar are feeding habits, niche habits, protective habits, reproductive habits and to what extent adaptive structures are developed. The last, in the writer's opinion, is still of rare value; while habits are of dominant value even in intergeneric competition.

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OBSERVATIONS ON THE MANNER OF CLASP- ING THE HANDS AND FOLDING THE ARMS

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LUTZ was the first to point out that individuals vary in the manner in which they clasp their hands. Thus, if individuals clasp their hands in such a manner that fingers of the right and left hands alternate, it will be found that approximately half of them will have the right thumb uppermost, whereas half will have the left thumb uppermost. Lutz failed to find any correlation between the manner of clasping the hands and right- or left-handedness. He did find, however, that there was a slight predominance of individuals with the right-handed hand-clasps in $R \times R$ matings, and a slight predominance of individuals with left-handed hand-clasps in $L \times L$ matings. This Lutz considered to be evidence that the manner of clasping the hands is a hereditary trait, although he could not state the exact mode of inheritance.

Downey studied the manner of clasping the hands in 1,040 men and 541 women. She found that 49 per cent. of the men and 54.2 per cent. of the women place the right thumb outside. She also found a certain tendency for left-handed people to place the left thumb uppermost when clasping the hands. Thus, of 571 right-handed men 51 per cent. had a right-handed clasp, whereas only 37.4 per cent. of 131 left-handed men had a right-handed clasp. Similarly, 56.2 per cent. of 333 right-handed women, whereas only 46.2 per cent. of 91 left-handed women placed the right thumb uppermost.

In 1930, I visited 131 families with 642 children, my main purpose at that time being to obtain blood for a study of the heredity of the agglutinogens M and N of Landsteiner and Levine. On most of these families,

observations were also made on the manner of clasping the hands, 120 families with 469 children being thus studied. In 105 families the manner of folding the arms was also observed. As a result of this investigation the following conclusions were reached:

(1) About 50 per cent. of individuals prefer to clasp their hands with the right thumb uppermost, whereas the remainder have the left thumb uppermost. (The actual percentages in a series of 709 individuals were 53.1 per cent. with right-handed hand-clasps and 46.9 per cent. with left-handed hand-clasps). The preference is a marked one, so that the alternative position feels uncomfortable. There are a few individuals, however, to whom the manner of clasping the hands is indifferent.

(2) Similarly, about 50 per cent. of individuals fold their arms with the right arm uppermost, whereas 50 per cent. prefer to have the left arm uppermost. (The actual percentages in a series of 595 individuals were 44.4 per cent. and 55.6 per cent., respectively.)

(3) The manner of folding the hands and arms are constant traits of each individual, as was shown by re-examination of 22 individuals after a period of 18 months.

(4) These traits are not inherited; neither are they associated with right- and left-handedness or sex.

(5) Marked preference with regard to the manner of crossing the knees does not exist.

(6) These observations would indicate that the manner of folding the hands and arms are habits formed by each individual early in life, which remain constant throughout life. The particular manner selected by each individual seems to be a matter of chance only.

In Table I is presented the results of observations on 120 families with 469 children with regard to the manner of clasping the hands. It may be seen that for the general population of 709 individuals, 53.1 per cent. have a right hand-clasp, and 46.9 per cent. have a left hand-clasp. By the χ^2 method of testing goodness of fit that

TABLE I
FAMILY DATA ON THE MANNER OF FOLDING THE HANDS

Father's hand clasp	Mother's hand clasp	Number of families	Children's hand clasp				Totals
			Right-handed clasp		Left-handed clasp		
			Male	Female	Male	Female	
Right	Right	36	34	42	32	34	142
Right	Left	32	30	32	30	20	112
Left	Right	29	30	39	24	29	122
Left	Left	23	22	20	28	23	93
Totals—Children			116	133	114	106	469
Parents			68	65	52	55	240
Grand Totals			382		327		709
Per cent.			53.1		46.9		

has been worked out by Pearson and Fisher, it is possible to determine whether or not the manner of clasping the hands has any correlation with sex, and whether or not it is hereditary. The lack of correlation with sex is proved in Table II, and the absence of heredity and

TABLE II
PROOF OF ABSENCE OF CORRELATION BETWEEN MANNER OF CLASPING THE
HANDS AND SEX

		Right-handed clasp		Left-handed clasp		n	χ^2	P
		Male	Female	Male	Female			
Parents	{ Actual	68	65	52	55	2	0.57	0.75
	{ Calculated	64	64	56	56			
Children	{ Actual	116	133	114	106	2	0.87	0.60
	{ Calculated	122	127	108	112			

correlation with sex in Table III. The method of calculation is very simple. Thus, in Table II, the calculation of χ^2 for the children is as follows:

For the 230 male children, the expectancies of 53.1 per cent. right hand-clasps and 46.9 per cent. left hand-clasps correspond to the approximate values 122 and 108, respectively.

Similarly, the corresponding expectancies for the 239 female children are 127 and 112, respectively.

Therefore, since

$$\chi^2 = \sum \frac{(x - x_o)^2}{x_o}$$

$$\text{Or } \chi^2 = \frac{(122 - 116)^2}{122} + \frac{(133 - 127)^2}{127} + \frac{(114 - 108)^2}{108} + \frac{(112 - 106)^2}{112}.$$

$$\chi^2 = 0.87.$$

And $n = 2$.

Then, from the table in Fisher's book: $P = 0.60$.

TABLE III
PROOF OF NON-HEREDITY OF CLASPING HANDS, AND LACK OF
CORRELATION WITH SEX

Cross		Children				n	χ^2	P
		Right-handed clasp		Left-handed clasp				
		Male	Female	Male	Female			
R \times R	{ Actual	34	42	32	34	2	0.29	0.88
	{ Calculated	35	40	31	32			
R \times L	{ Actual	30	32	30	20	2	1.85	0.40
	{ Calculated	33	28	27	24			
L \times R	{ Actual	30	39	24	29	2	0.58	0.75
	{ Calculated	29	36	25	32			
L \times L	{ Actual	22	20	28	23	2	2.85	0.25
	{ Calculated	27	23	23	20			

TABLE IV
FAMILY DATA ON THE MANNER OF FOLDING THE ARMS

Father	Mother	Number of families	Children				Totals
			Right		Left		
			Male	Female	Male	Female	
Right	Right	25	14	17	26	24	81
Right	Left	29	24	28	31	23	106
Left	Right	15	15	14	21	24	74
Left	Left	34	26	32	37	33	128
Totals—Children			79	91	115	104	339
Parents			54	40	49	53	206
Grand totals			264		331		595
Per cent.			44.4		55.6		

In Tables IV, V, and VI is shown in a similar manner

TABLE V
PROOF OF ABSENCE OF CORRELATION BETWEEN MANNER OF FOLDING
ARMS AND SEX

		Right arm upper- most		Left arm upper- most		n	χ^2	P
		Male	Female	Male	Female			
Parents	{ Actual	54	40	49	63	2	3.92	0.15
	{ Calculated	46	46	57	57			
Children	{ Actual	79	91	115	104	2	1.35	0.50
	{ Calculated	86	87	108	108			

TABLE VI
PROOF OF NON-HEREDITY OF MANNER OF FOLDING ARMS, AND LACK OF
CORRELATION WITH SEX

Cross		Children				n	χ^2	P
		Right arm upper- most		Left arm upper- most				
		Male	Female	Male	Female			
R × R	{ Actual	14	17	26	24	2	1.02	0.60
	{ Calculated	17	18	23	23			
R × L	{ Actual	24	28	31	23	2	1.93	0.40
	{ Calculated	24	23	31	28			
L × R	{ Actual	15	14	21	24	2	1.07	0.60
	{ Calculated	16	17	20	21			
L × L	{ Actual	26	32	37	33	2	0.81	0.65
	{ Calculated	28	29	35	36			

that no correlation exists between the manner of folding the arms and sex, and that there is no heredity of this trait.

There are four possible combinations of the manner of clasping the hands with the manner of folding the arms. Thus, individuals who clasp their hands with the right thumb uppermost may fold their arms with either the right or the left arm on top; and the same statement holds for individuals with a left hand-clasp. These four classes may be designated as RR, RL, LR, and LL, re-

spectively, where the first letter represents the type of hand-clasp, and the second letter the manner of folding the arms. Of 219 parents, there were 51 of type RR, 64 RL, 47 LR, and 57 LL. For the frequencies of right and left hand-clasps given in Table I, and the frequencies of right and left arm-folds given in Table IV, the expectancies are 51 RR, 65 RL, 45 LR, and 58 LL, if there is no correlation between the two traits. Therefore, $\chi^2 = 0.121$, and since $n = 3$, $P = 0.99$.

Twenty-two individuals were revisited after a period of 18 months. Not one of them showed any change in the manner of clasping the hands. Three individuals, however, did not show any particular preference in the manner of folding the arms, which they varied from time to time.

Thirty-four left-handed individuals were examined. Of these there were 5 RR, 7 RL, 12 LR, and 10 LL. When we compare these frequencies with the frequencies in the general population (51 RR, 64 RL, 47 LR, and 57 LL) we find $\chi^2 = 4.10$; and since $n = 3$, $P = 0.25$, so that there is no apparent correlation with right- or left-handedness.

SUMMARY AND CONCLUSIONS

A large series of families was studied with reference to the manner of clasping the hands and folding the arms. The results indicate that these traits are not hereditary, show no correlation with sex, handedness or one another. They are probably habits formed early in life, which then remain constant throughout life.

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SHORTER ARTICLES AND DISCUSSION

SHELL GROWTH IN THE PERIWINKLE, *LITTORINA LITOREA*

THE formula, $y = bx^k$, where y is a linear measurement or weight of a part and x that of the whole while b and k are constants, has been used by Huxley and his students (1927, 1931) and others to describe differential growth in a number of forms, including molluses, crustaceans, insects and mammals. When k equals unity, the part bears a constant relation to the whole and its proportion is unchanged with increasing size. This type is termed by Huxley "isogonic growth." When k is greater or less than unity the part increases or decreases relatively with increased size of the whole. These situations he terms "positive" and "negative heterogony." The magnitude of the constants are influenced by the part and the organism investigated while b , in addition, is of course dependent on the unit of measure employed. Usually, in positively heterogonic growth, k is less than 2, although as in the case of the female pea-crab (*Pinnotheres pisum*) it may greatly exceed the figure (Huxley, 1931). The constant k , has been designated by Nomura (1926a) the "specific exponent," while he calls b the "local constant," since in the same species he found that it sometimes varied from locality to locality.

This general formula has also been utilized to describe accurately the weight-length relationship in several forms, especially fishes and molluses. When thus employed the value of k is usually greater than 3 but less than 4 (e. g., Clark, 1928, Galtsoff, 1931), although for some species of fishes the weight seems to increase directly as the cube of the length.

On September 21, 22 and 23, 1931, the authors collected a large series of the periwinkle, *Littorina litorea*, within an area of a few square rods in tide pools near Bar Harbor, Maine. This series ranged in length of shell from 5.82 mm to 31.6 mm, probably representing all classes but the very young animals. After the shells had been freed from their occupants, they were allowed to dry in the open air for several days before being measured and weighed. Two measurements were taken on each shell. The length was taken as the distance from the apex of

the spire to the most distant point on the outer lip. The diameter of the aperture was taken as the distance from the junction of the outer lip and the body whorl to the most distant point on the former. These measurements were taken with vernier calipers to the nearest .1 mm, except in the case of some of the smallest shells, which were measured with a bench micrometer to the nearest .01 mm. All shells were weighed to the nearest .01 gram.

Table I gives the distribution of the shells according to length, together with the mean weight and the mean diameter with their coefficients of variability, for each class.

TABLE I
BIOMETRICAL CONSTANTS OF *Littorina litorea* SHELLS

Length (mm)	No. of specimens	Mean weight (grams)	Coefficient of variation (per cent.)	Mean diameter of aperture (mm)	Coefficient of variation (per cent.)
4.0 - 5.9	1	.020		4.16	
6.0 - 7.9	6	.063		5.55	
8.0 - 9.9	11	.174 ± .011	29.89	7.14 ± .091	6.25
10.0 - 11.9	24	.271 ± .010	26.20	8.55 ± .077	6.51
12.0 - 13.9	20	.408 ± .011	18.38	9.74 ± .090	6.11
14.0 - 15.9	22	.745 ± .016	15.03	11.48 ± .061	3.68
16.0 - 17.9	38	1.021 ± .026	23.02	12.89 ± .085	6.00
18.0 - 19.9	37	1.637 ± .027	15.09	14.72 ± .067	4.12
20.0 - 21.9	46	2.154 ± .026	11.88	16.17 ± .071	4.41
22.0 - 23.9	74	2.831 ± .028	12.50	17.50 ± .054	3.93
24.0 - 25.9	52	3.394 ± .036	11.34	18.55 ± .054	3.09
26.0 - 27.9	24	4.217 ± .049	8.39	19.37 ± .064	2.40
28.0 - 29.9	3	5.317		21.03	
30.0 - 31.9	2	6.570		21.95	

Although there is a considerable variation in weight in each length class, the mean weight increases at a definite rate with increased length. When the logarithms of mean weight are plotted against the logarithms of length, as shown in Fig. 1, the result, with the exception of the first class which contains but a single individual, is approximately a straight line. The relationship between weight and length, therefore, can be expressed by the formula, $y = bx^k$. Determining the constant b and

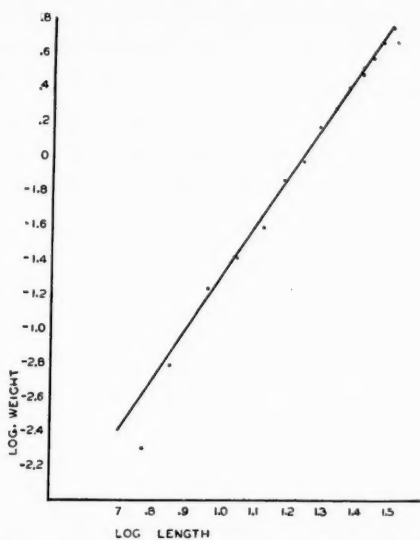


FIG. 1. Relationship between log. of weight and log. of length of shells.

k, by the method of least squares (omitting the two shortest and the two longest classes because of their small numbers), we obtain

$$\text{Weight} = .000194 \text{ Length}^{3.04160}$$

The curve of this equation is shown in Fig. 2, while the

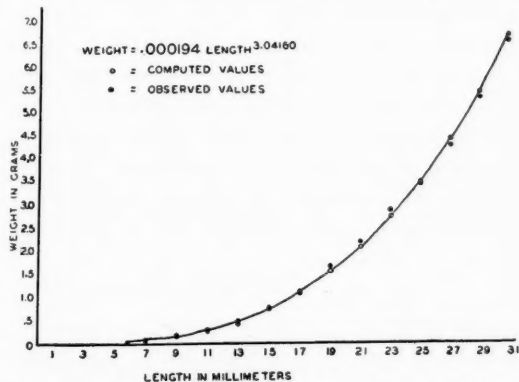


FIG. 2. Relationship between weight and length of shells.

calculated and observed weights are listed in Table II. It can be seen that weights computed from this formula fit the observed means very closely. Our value of k in the weight-length relationship of *Littorina litorea* shells is rather lower than that found for the bivalve, *Sphaerium heterodon*, by Nomura (1926a) or for the Hawaiian pearl oyster by Galtsoff (1931). In both these cases the value of the exponent was slightly in excess of 3.2.

TABLE II
OBSERVED AND CALCULATED VALUES OF THE SHELLS OF *Littorina litorea*

Length (mm)	No. of specimens	Observed mean weight (gms)	Calculated weight (gms)	Difference between calculated and observed weight	Observed diameter of body whorl (mm)	Calculated diameter of aperture (mm)	Difference between calculated and observed diameter
4.0 - 5.9(5.82)	1	.020	.040	+.020	4.16	4.42	+.26
6.0 - 7.9	6	.063	.072	+.009	5.55	5.32	-.23
8.0 - 9.9	11	.174	.155	-.019	7.14	6.84	-.30
10.0 - 11.9	24	.271	.285	+.014	8.55	8.36	-.19
12.0 - 13.9	20	.408	.474	+.066	9.74	9.89	+.15
14.0 - 15.9	22	.745	.733	-.012	11.48	11.41	-.07
16.0 - 17.9	38	1.021	1.072	+.051	12.89	12.93	+.04
18.0 - 19.9	37	1.637	1.504	-.133	14.72	14.45	-.27
20.0 - 21.9	46	2.154	2.039	-.115	16.17	15.97	-.20
22.0 - 23.9	74	2.831	2.689	-.142	17.50	17.50	0
24.0 - 25.9	52	3.394	3.446	+.052	18.55	19.02	+.47
26.0 - 27.9	24	4.217	4.380	+.163	19.37	20.54	+1.17
28.0 - 29.9	3	5.317	5.443	+.126	21.03	22.06	+1.03
30.0 - 31.9	2	6.570	6.667	+.097	21.95	23.59	+1.64

The relation between the diameter of the aperture and the length of the shell can likewise be expressed by the same general formula. Between the lengths of 8.0 mm and 27.9 mm inclusive we obtain the following formula by the method of least squares:

$$\text{Diameter} = .75940 \text{ Length}^{1.00055}$$

This curve is depicted in Fig. 3, while the observed and calculated means are given in Table II. From this formula it is obvious that the growth in diameter of the aperture is approxi-

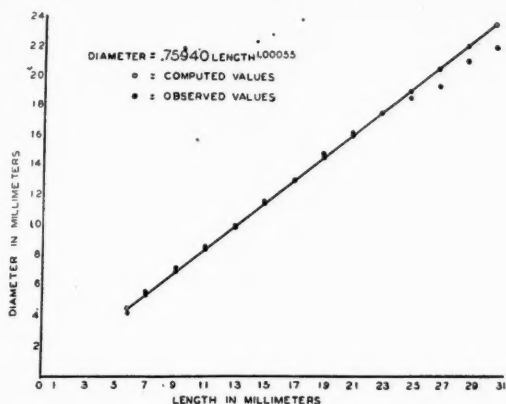


Fig. 3. Relationship between diameter or aperture and length of shells.

mately isogonic, although there seems to be a tendency for the computed diameters of the longest shells to exceed the observed. The two extreme classes, however, contain so few individuals that little reliance can be placed in them.

In most molluscan species which have been investigated linear relationships of the shells (height-width, width-length) are heterogonic, (Nomura, 1926, 1926a; Sasaki, 1926; Weymouth and McMillin, 1931) although in two gasteropods, Nomura (1926a) found the growth of height in relation to width to be isogonic.

The data on our series of shells of *Littorina litorea* show that the growth relation between weight and length can be expressed by the formula, $y = bx^k$, with the value of the exponent 3.0416.

It has also been determined that in our series the growth relation between the diameter of the aperture and the length of the shell is isogonic.

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VESTIGIAL CLAVICLES AND RUDIMENTARY SESAMOIDS¹

THEIR DEVELOPMENT AND FUNCTIONS IN MAMMALS

It is interesting to observe the presence or absence of the clavicle, or collar-bone, in different groups of Mammalia, and the various stages to be found between a fully functional bone and a non-functional vestige.

While the clavicles in the Canidae have been known to anatomists, it is almost an unheard-of thing to find them in a mounted dog or wolf skeleton, yet it is safe to say that they are never absent in the living subject, although extremely small and quite without function. They have no articulation with any part of the skeleton, but are simply lodged among the muscles of the shoulders, there being no bone connection between the foreleg and the body. The scapula, or shoulder-blade, with which the foreleg articulates, is connected with the rest of the skeleton only by muscles and tendons.

¹ Photographs by the author.

In a large-sized dog skeleton, the Russian wolfhound, which has recently been mounted and placed on exhibition in the American Museum of Natural History, I find the clavicles roughly almond shaped, measuring 4 mm wide at their greatest vertical diameter, and 10 mm in length; while in a whippet, a small racing dog standing about 37 meters high at the shoulders, the clavicles measure only 2 by 3 mm.

It would appear that the Felidae must have found some use for this organ at a much later period than did the Canidae, as the cat family possesses quite a substantial looking clavicle, that of a lion being 7 mm in vertical diameter, and 73 mm long, a bone of sufficient size to demand the attention of the most careless observer, but one which is of very little, if any, service to its possessor at the present time.

A rather undersized raccoon skeleton, also on exhibition at the museum, shows a pair of clavicles which in form are curved shafts, measuring 1 by 8 mm. Here again we have vestiges handed down from the past, which have no function at the present time.

It will probably be found, upon investigation, that the clavicles are present in a more or less vestigial form in all the Carnivora.

The horses, asses and zebras (Equidae) and the cud-chewing animals (Ruminants) are without clavicles and so far as is known all the hoofed animals (Ungulates), but it does not necessarily follow that a remaining vestige may not yet be discovered in some species.

The clavicle is well developed and highly functional in man, in the bats (Chiroptera), and is present in a functional form in most of the gnawing animals (Rodentia). It is well developed in all the pouched animals (Marsupialia) except the bandicoots (Paramelidae).

One of the important functions of the clavicle is to furnish a fulcrum upon which the pectoral muscles are exerted in effecting a strong movement of the manus, or hand, toward the median line, as is so characteristic in man, where this bone is still very strong and well developed. In this respect, the human subject has remained primitive. The massive pectoral muscle, with its origin on the sternum and insertion near the head of the humerus, and the reverse action of the trapezius, deltoid and infraspinatus muscles of the back and shoulder give the human arm a

great range of lateral movement. This is admirably illustrated when the lumberman in a northern camp drops his ax for a moment while he exercises his arms, thrashing his hands against his shoulders to drive the circulation and warmth to his cold finger tips.

In the Equidae, on the other hand, with their highly specialized limbs, where the action is restricted to a simple back-and-forth movement, there is not the same necessity for a clavicle.

In their evolution, the clavicles are of very early origin, and, in animals where they are still highly functional, their calcification begins at a surprisingly early period in the foetal development of the individual.

While, in a great many mammals, the clavicles are now in the last stage of a slow decline, the sesamoid bones, as a class, are in their early infancy and may be regarded as of comparatively recent origin. With the exception of the human subject, where there are but few sesamoids, they are very prevalent among the higher mammals. They occur less frequently in the more primitive mammals and are even fewer in number in the reptiles.

These bones are always formed in a tendon, from a single ossification center, at a point where a tendon passes over an articular surface where there is great strain, and violent action to be performed. The sesamoid, with its articular cartilage and lubricant, saves wear on the tendon and reduces friction, thus conserving power. The oldest and most highly developed and perhaps the most useful sesamoid in the system is the patella, or knee-cap, at the knee joint.

In most cases these bones appear to be speed adaptations, while in others they have been developed where a comparatively slow but strong movement is required, as for example in the aard-vark (*Orycteropus capensis*), an animal of no speed but most efficient in excavating great burrows in the ground. This peculiar South African animal has many highly developed sesamoids.

The Canidae are unusually prolific in the production of sesamoid bones, there being from sixty-two to seventy-eight and occasionally eighty-two. Many of these may not be considered as rudimentary, but are well developed. Eight are located at the knee joints, the others on the feet. On the palmar surface of the metapodials there is a set of forty of these bones. These are well developed and are present in most mammals.

The more rudimentary sesamoids are at the distal ends of the metapodials on the dorsal surface, as seen in the illustration on p. 1. Then, similarly situated on the distal ends of the proximal

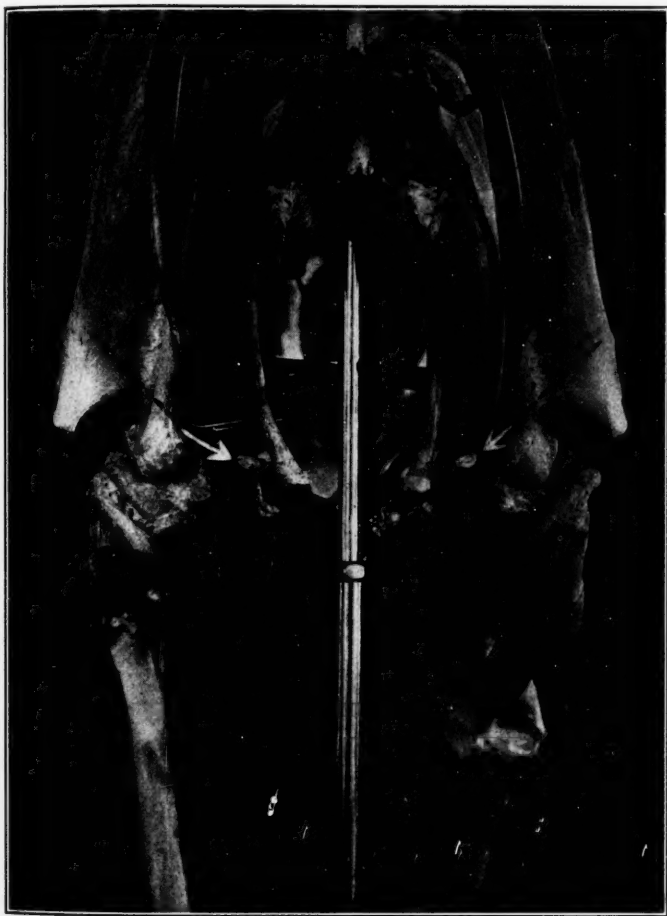


FIG. 1. Front view of a wolfhound's skeleton. The arrows point to very small vestigial clavicles.

phalanges, the middle joints of the toes, we find another set even more rudimentary and irregular in their occurrence. These of

the second set range from 3 mm to a size almost microscopic, or are frequently absent, so that it is not surprising that they have been so generally overlooked in the dissection and preparation of specimens. They are particularly interesting because they



FIG. 2. The bones of a wolfhound's foot. The arrows point to the rudimentary sesamoids.

are not generally known, although some of them were figured, by De Blainville, as long ago as 1839.

All these sesamoids that occur on the dorsal surface of the joints of the feet are, undoubtedly, of much more recent origin than any of the others and, we may assume, are continuing in their development, thus adding still further to the speed mechanism of these swift-footed animals.

Many of the sesamoids, particularly those that are more advanced in their development, are fairly well formed before birth; as it were, in anticipation of the hard work that they will be called upon to perform later, when their possessor is fleeing from a predaceous enemy, or is acting the part of the pursuer, or digging a hole in the ground to be used as he may find expedient.

S. HARMSTED CHUBE

AMERICAN MUSEUM OF NATURAL HISTORY

THE RÔLE OF MALE PARTHENOGENESIS IN THE EVOLUTION OF THE SOCIAL HYMENOPTERA

IN the whole animal kingdom no other order can compare with the Hymenoptera in the number and diversity of its social species. In the whole animal kingdom, with a few notable exceptions, the production of males from unfertilized eggs is confined to the Hymenoptera. Is there an important causal relationship between these two facts? A consideration of the genetic consequences of male parthenogenesis, especially when acting in conjunction with another characteristic of most species of Hymenoptera, namely, the marriage flight, makes an affirmative answer to this question probable. Male parthenogenesis plus the marriage flight acts as an efficient eugenic contrivance ensuring the Hymenopteran colony a freedom from unfit individuals which is a marked advantage in competition with insect colonies of other species. Male parthenogenesis is a valuable adaptation to a social existence.

The marriage flight, besides its function of permitting crossing between colonies, serves as a selective contrivance by preventing unfit males from mating with the queen. Since the mating occurs high in the air, only sound males with good powers of flight can achieve it; individuals with defective wings or eyes, or any one of a variety of other possible hereditary defects, are eliminated as potential sires of the next generation of workers. A phenotypically sound father is thus guaranteed to the colony.

This alone, however, is not sufficient to ensure the transmission of a good heredity. It is only because the male is haploid that the selective action of the marriage flight is fully effective. In haploid individuals each chromosome and each gene is represented but once, not twice as in the more common diploid forms. Hence in the formation of germ cells there can be no segregation;

but one type of sperm is produced. The genotype is identical with the phenotype and every individual breeds as he appears. Thus the phenotypically sound male selected by the marriage flight to sire the next generation of workers can produce only genetically sound sperm. Moreover, since good traits are usually dominant, the workers in most cases will be without defects even when harmful mutations have segregated in the eggs produced by the queen. The selective action of the marriage flight is thus a good guarantee of physically sound progeny.

This high efficiency of selection characteristic of the Hymenoptera stands in marked contrast to the comparatively low effectiveness of selection in diploid species. Here a pair selected as phenotypically perfect may carry an indefinite number of harmful mutant genes capable of cropping out in their progeny. Congenitally defective young from healthy parents due to this cause are relatively common in most species of animals from *Drosophila* up to and including man. Probably even more common is the death of embryos due to the action of lethal genes.

Consider the consequences to, let us say, the honey bee, where both parents diploid and hence capable of transmitting harmful recessive factors. In many, perhaps in the majority of hives, one fourth or more of the workers would manifest some imperfection or die without emerging from the comb. This would mean at the least the waste of much valuable comb space, and might involve the support by the rest of the hive of defective individuals incapable of carrying their share of the hive's labors. Any colony thus affected would be at an obvious disadvantage in the struggle for existence. As the Hymenoptera are constituted, however, such a situation is practically impossible. The eugenic efficiency of their method of reproduction ensures a sound heredity for the great majority of colonies.

There is a second interesting genetic consequence of male parthenogenesis which may have played some rôle in the evolution of the Hymenoptera. This is the uniformity which it causes in all the diploid individuals of a colony. Since the male, being haploid, produces only one kind of spermatozoon, his daughters are identical with respect to at least half of their germ-plasm. They are not only all sisters, they are half way to being identical twins. As already pointed out, moreover, the traits of the male are more likely than not to be dominant, and this will further accentuate the uniformity of his progeny. The similarity of all

workers from one father may partly account for the ability of ants to recognize members of their own colony. Uniformity of instincts, too, may well be of value as a factor favorable to cooperative effort in a social existence.

While the method of sex determination and reproduction found in the Hymenoptera is perhaps as well adapted to the elimination of congenital defects as any that could be devised, there are yet certain conditions in which defective individuals may be expected to appear. Though it usually guarantees a good heredity for the females, it permits a high incidence of defectives among the males. If a queen is heterozygous for a harmful recessive gene, as must occasionally be the case, half her sons will be defective. The loss that might result from this has been met in the bees and ants by reducing the number of males and assigning them no part in the economy of the hive or colony. They are discarded after mating is accomplished. Besides the defective males, occasional defective females will probably appear. These may be due to three causes. First, a mutant spermatozoon may unite with an egg carrying the same mutation. This must be a very rare occurrence. Second, the queen may be heterozygous for a harmful gene that is not entirely recessive. Such genes, while not common, are by no means unknown in most species that have been studied. Should the queen carry such a one, it would reappear in half the workers. Third, there probably exist sex-limited mutations which do not express themselves in males, but only in workers or queens. A mutation of this type, acting on the maturation of the eggs, may have caused the few cases where a considerable proportion of the workers in a hive have been gynandromorphs.

Despite these loopholes through which imperfect germ-plasm can escape the action of selection, the manner of reproduction prevailing in the Hymenoptera stands unexcelled in its eugenic efficiency. Only among the termites has there been evolved a system that is at all comparable. In colonies of these remarkable insects the defective young are eaten by their more healthy brothers and sisters.¹ Thus a certain proportion of defective germ-plasm is continually eliminated, and no burden of termite incompetents is carried by the colony. Some waste is involved, however, in rearing the young to the point where defects appear;

¹ W. M. Wheeler, 1928. "Foibles of Insects and Men," pp. 212-215. New York: Alfred A. Knopf.

that system is better in which no defective individuals can be born. It is a fact too striking to be a coincidence that the termites, which alone among insects can rival the Hymenoptera in the complexity of their societies, are, also like the Hymenoptera, endowed with a eugenic system of high efficiency. The Hymenoptera have the advantage, however, that they were endowed with their system from the start, for male parthenogenesis must have appeared very early in their evolution. This is not the only characteristic which peculiarly fits them to a social life,² but it is an important and unique one, and it may be regarded as among the major adaptations which have led to the development of so many social species in this order.

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² F. M. Wheeler, 1928. "The Social Insects," p. 130. New York: Harcourt, Brace and Company.

